Access Db# 145335

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: MARCELA M CORDERO GARCIA Examiner # : 80381 Date: 2/16/05
Art Unit: 1654 Phone Number 3/1 2-2939 Serial Number: 10/726,919
Mail Box and Bldg/Room Location: REM 3C18 / 3C35 Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need. ME

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Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: DETERMINATION OF TESTOSTERONE BY MASS SPECTROMETRY

Inventors (please provide full names): SEE BIB DATA ATTACHD

Earliest Priority Filing Date: SEE BIB DATA SHEET

**For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

PLEASE SEARCH CLM 1

THANKS

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STAFF USE ONLY	Type of Search	Vendors and cost where applicable
Searcher:	NA Sequence (#)	STN
Searcher Phone #:	AA Sequence (#)	Dialog
Searcher Location:	Structure (#)	Questel/Orbit
Date Searcher Picked Up:	Bibliographic	Dr.Link
Date Completed:	Litigation	Lexis/Nexis
Searcher Prep & Review Time:	Fulltext	Sequence Systems
Clerical Prep Time:	Patent Family	WWW/Internet
Online Time:	Other	Other (specify)

PTO-1590 (8-01)

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     FILE 'HCAPLUS' ENTERED AT 11:25:38 ON 18 FEB 2005
                  E CAULFIELD MICHAEL P/AU
L17             45 SEA ABB=ON  "CAULFIELD MICHAEL P"/AU
                  E CARNS DARREN A/AU
                  E REITZ RICHARD E/AU
L18              7 SEA ABB=ON  "REITZ RICHARD E"/AU
L19              1 SEA ABB=ON  L17 AND L18
                  D TI
L20             51 SEA ABB=ON  L17 OR L18
L21              0 SEA ABB=ON  L20 AND ?TESTOST?
L22                ANALYZE L19 1-1 CT :       5 TERMS

     FILE 'REGISTRY' ENTERED AT 11:31:25 ON 18 FEB 2005
                  E TESTOSTERONE/CN
L23              1 SEA ABB=ON  TESTOSTERONE/CN

     FILE 'HCAPLUS' ENTERED AT 11:31:40 ON 18 FEB 2005
L24            970 SEA ABB=ON  (L23 OR ?TESTOSTERON?) AND ?MASS?(W)?SPECT?
L25            822 SEA ABB=ON  L24 AND (?DETECT? OR ?DETERMIN? OR ?ANAL?)
L26              0 SEA ABB=ON  L25 AND (?HIGH?(W)?TURBIN?(W)?LIQUID?(W)?CHROMATOG?
                   OR ?HTLC?)
L27            177 SEA ABB=ON  L25 AND LIQ?(W)?CHROMATOG?
L28             23 SEA ABB=ON  L27 AND ?PURIF?
L29             23 SEA ABB=ON  L28 AND (PRD<20031202 OR PD<20031202)
                  SAV L29 COR919L29/A

     FILE 'MEDLINE, BIOSIS, EMBASE, JAPIO, JICST-EPLUS' ENTERED AT 11:36:04 ON
     18 FEB 2005
L30             62 SEA ABB=ON  L29
L31             49 DUP REMOV L30 (13 DUPLICATES REMOVED)
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23 cits from CAPlus

49 cits from other databases

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=> d que stat l29
L23           1 SEA FILE=REGISTRY ABB=ON  TESTOSTERONE/CN
L24         970 SEA FILE=HCAPLUS ABB=ON  (L23 OR ?TESTOSTERON?) AND ?MASS?(W)?S
                PECT?
L25         822 SEA FILE=HCAPLUS ABB=ON  L24 AND (?DETECT? OR ?DETERMIN? OR
                ?ANAL?)
L27         177 SEA FILE=HCAPLUS ABB=ON  L25 AND LIQ?(W)?CHROMATOG?
L28          23 SEA FILE=HCAPLUS ABB=ON  L27 AND ?PURIF?
L29          23 SEA FILE=HCAPLUS ABB=ON  L28 AND (PRD<20031202 OR PD<20031202)

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L29  ANSWER 1 OF 23  HCAPLUS  COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER:        2003:738884  HCAPLUS
DOCUMENT NUMBER:         139:302164
TITLE:                   Neurosteroids in rat brain: extraction, isolation, and
                         anal. by nanoscale liquid
                         chromatog.-electrospray mass
                         spectrometry
AUTHOR(S):               Liu, Suya; Sjoevall, Jan; Griffiths, William J.
CORPORATE SOURCE:        Department of Medical Biochemistry and Biophysics,
                         Karolinska Institutet, Stockholm, SE-171 77, Swed.
SOURCE:                  Analytical Chemistry (2003), 75(21),
                         5835-5846
                         CODEN: ANCHAM; ISSN: 0003-2700
PUBLISHER:               American Chemical Society
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   A method designed for the anal. of sulfated neurosteroids and
     unconjugated ketonic neurosteroids in rat brain using nanoscale
     liquid chromatog.-electrospray (nano-LC-ES) mass
     spectrometry is described. Neurosteroids in rat brain tissue were
     extracted, purified, and separated into two groups, neutral unconjugated
     steroids and steroid sulfates, by employing solid-phase partition, cation-
     and anion-exchange chromatog. The steroid sulfate fraction was
     analyzed by nano-LC-ES mass spectrometry.
     Contrary to expectations, the sulfates of pregnenolone and
     dehydroepiandrosterone (DHEA) were not detected. Internal
     stds., including pregnenolone sulfate, were recovered and the
     detection limit of the method was 0.3 ng/g of wet brain.
     Cholesterol sulfate was detected at a level of 1.2 μg/g of
     wet brain. The neutral unconjugated steroid fraction was derivatized with
     hydroxylamine hydrochloride to convert oxosteroids into their oximes. The
     oximes were isolated using cation-exchange chromatog. and were
     analyzed by nano-LC-ES tandem mass spectrometry
     . The analyses of the neutral unconjugated steroid fraction
     confirmed the presence in rat brain of pregnenolone, pregnanolone isomers,
     progesterone, testosterone, and DHEA, which were characterized
     by their retention times, the mass of the protonated mols., and
     characteristic fragment ions. The levels were estimated by addition of
     [3,4-13C2]-progesterone as an internal standard and found to be in a range of
     0.04-20 ng/g.
REFERENCE COUNT:         36    THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS
                               RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29  ANSWER 2 OF 23  HCAPLUS  COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER:        2003:345242  HCAPLUS
DOCUMENT NUMBER:         139:207898
TITLE:                   Confirmatory analysis of 17β-boldenone,
                         17α-boldenone and androsta-1,4-diene-3,17-dione
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in bovine urine by **liquid chromatography**-tandem **mass spectrometry**
AUTHOR(S): Draisci, Rosa; Palleschi, Luca; Ferretti, Emanuele; Lucentini, Luca; delli Quadri, Fernanda
CORPORATE SOURCE: Laboratorio di Medicina Veterinaria, Istituto Superiore di Sanita, Rome, 00161, Italy
SOURCE: Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences (**2003**), 789(2), 219-226
CODEN: JCBAAI; ISSN: 1570-0232
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A sensitive and selective **liquid chromatog.**-tandem **mass spectrometry** (LC-MS-MS) method for confirmatory **anal.** of 17β-boldenone (17β-BOL), 17α-boldenone (17α-BOL) and androsta-1,4-diene-3,17-dione (ADD) in bovine urine was developed. [2H2]17β- **Testosterone** (17β-T-d2) was used as the internal standard Sample preparation involved enzymic hydrolysis and **purification** on a C18 solid-phase extraction column. Chromatog. separation of the **analytes** was obtained using an RP-C18 HPLC column. LC-MS-MS **detection** was carried out with an atmospheric pressure chemical ionization (APCI) source equipped with a heated nebulizer (HN) interface operating in the pos. ion mode. For unambiguous hormone confirmation, three **analyte** precursor-product ion combinations were monitored during multiple-reaction monitoring (MRM) LC-MS-MS **anal.** Overall recovery (%), repeatability (relative standard deviations, RSD, %) and within-laboratory reproducibility (RSD, %) ranged from 92.2 to 97.7%, from 6.50 to 2.94% and from 13.50 to 5.04%, resp., for all **analytes.** The limit of quantification in bovine urine was 0.20 ng ml-1 for 17β-BOL and ADD and 0.50 ng ml-1 for 17α-BOL. The validated method was successfully applied for **determination** of 17β-BOL, 17α-BOL and ADD in a large number of bovine urine samples collected within the national Official Residue Control Program.
REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 3 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:313406 HCAPLUS
DOCUMENT NUMBER: 139:67981
TITLE: Screening for gestagens in kidney fat using accelerated solvent extraction and **liquid chromatography** electrospray tandem **mass spectrometry**
AUTHOR(S): Hooijerink, H.; van Bennekom, E. O.; Nielen, M. W. F.
CORPORATE SOURCE: State Institute for Quality Control of Agricultural Products, Wageningen, 6700 AE, Neth.
SOURCE: Analytica Chimica Acta (**2003**), 483(1-2), 51-59
CODEN: ACACAM; ISSN: 0003-2670
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A screening method was developed for the **determination** of various anabolic steroids in kidney adipose tissue. Fatty tissue samples are extracted and the steroids were trapped online during accelerated solvent extraction (ASE). Following this initial extraction the samples were further **purified** by C18 solid-phase extraction (SPE). The steroid compds. were

finally **analyzed** by HPLC-tandem MS. The method was validated using blank kidney fat and fat samples spiked at 2 ng/g. The **detection** capability (CCβ) was <2 ng/g and the decision limit (CCα) varied 0.3-0.9 ng/g. The recoveries were 17-58% and the accuracy using isotopically-labeled internal stds. varied 100-135%. The method was successfully used in the screening for the gestagens flurogestone acetate (FGA), delmadinone acetate (DMA), megestrol acetate (MEGA), chloromadinone acetate (CMA), melengestrol acetate (MELA), and medroxyprogesterone acetate (MPA), and for the androgen **chlorotestosterone** acetate (CTA) in kidney fat.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 4 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:145650 HCAPLUS
DOCUMENT NUMBER: 138:296821
TITLE: Polar derivatization of 5α-**dihydrotestosterone** and sensitive **analysis** by semimicro-LC/ESI-MS
AUTHOR(S): Nakagawa, Yumiko; Hashimoto, Yutaka
CORPORATE SOURCE: Pharmaceuticals Group, Nippon Kayaku Co., Ltd., Kita-ku, Tokyo, 115-8588, Japan
SOURCE: Journal of the Mass Spectrometry Society of Japan (**2002**), 50(6), 330-336
CODEN: JMSJEY; ISSN: 1340-8097
PUBLISHER: Nippon Shitsuryo Bunseki Gakkai
DOCUMENT TYPE: Journal
LANGUAGE: Japanese

AB Polar derivatization method for electrospray ionization **mass spectrometry** (ESI-MS) was developed and applied to the sensitive **anal.** of 5α- **dihydrotestosterone** (DHT) in prostate and plasma. Rat prostate was dissolved with alkaline solution and DHT in the prostate was extracted by solid phase extraction, and derivatized to the N-methylpyridinium-DHT as a polar derivative The N-methylpyridinium derivative of DHT was **purified** by solid-phase extraction using Bond Elut C18, and **determined** by a semimicro-LC/ESI-MS with selected reaction monitoring (SRM). Linear responses of the N-methylpyridinium derivative of DHT were observed for **analyte** concentration from 5 to 100 pg/tube. DHT values were 12.0 ± 3.8 ng/g tissue in rat prostates, 171.7 ± 127.2 pg/mL in rat plasma and 286.2 ± 123.5 pg/mL in human plasma.

L29 ANSWER 5 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:511044 HCAPLUS
DOCUMENT NUMBER: 137:320443
TITLE: Identification of dehydroepiandrosterone metabolites formed from human prostate homogenate using **liquid chromatography-mass spectrometry** and gas chromatography-**mass spectrometry**
AUTHOR(S): Mitamura, Kuniko; Nakagawa, Takashi; Shimada, Kazutake; Namiki, Mikio; Koh, Eitetsu; Mizokami, Atsushi; Honma, Seijiro
CORPORATE SOURCE: Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, 920-0934, Japan
SOURCE: Journal of Chromatography, A (**2002**), 961(1), 97-105
CODEN: JCRAEY; ISSN: 0021-9673
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal

LANGUAGE: English

AB The identification of the in vitro metabolites of dehydroepiandrosterone formed from human prostate homogenate was investigated by hyphenated techniques using the stable-isotope dilution method. A mixture of dehydroepiandrosterone and [2H4]dehydroepiandrosterone was incubated with hypertrophied human prostate tissue homogenate in the presence of NAD, NADH and NADPH. The metabolites were extracted with AcOEt-hexane, **purified** by solid-phase extraction, and then **analyzed** by LC-atmospheric pressure chemical ionization MS and/or GC-MS. Androst-5-ene-3β,17β-diol (major product), androst-4-ene-3,17-dione, **testosterone**, 5α- **dihydrotestosterone**, androsterone, and 7α-hydroxydehydroepiandrosterone were identified in comparison with authentic samples based on their chromatog. behavior and **mass spectra.**

REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 6 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:266898 HCAPLUS

DOCUMENT NUMBER: 136:380350

TITLE: Mechanism-based inactivation of cytochrome P450 3A4 by 17α-ethynylestradiol: Evidence for heme destruction and covalent binding to protein

AUTHOR(S): Lin, Hsia-Lien; Kent, Ute M.; Hollenberg, Paul F.

CORPORATE SOURCE: Department of Pharmacology, University of Michigan, Ann Arbor, MI, USA

SOURCE: Journal of Pharmacology and Experimental Therapeutics (**2002**), 301(1), 160-167
CODEN: JPETAB; ISSN: 0022-3565

PUBLISHER: American Society for Pharmacology and Experimental Therapeutics

DOCUMENT TYPE: Journal

LANGUAGE: English

AB 17α-Ethynylestradiol (EE), a major constituent of many oral contraceptives, inactivated the **testosterone** 6β-hydroxylation activity of **purified** P 450 3A4 reconstituted with phospholipid and NADPH-cytochrome P 450 reductase in a mechanism-based manner. The inactivation of P 450 3A4 followed pseudo first order kinetics and was dependent on NADPH. The values for the K1 and kinact were 18 μM and 0.04 min-1, resp., and the t1/2 was 16 min. Incubation of 50 μM EE with P 450 3A4 at 37° for 30 min resulted in a 67% loss of **testosterone** 6β-hydroxylation activity accompanied by a 35% loss of the spectral absorbance of the native protein at 415 nm and a 70% loss of the spectrally **detectable** P 450-CO complex. The inactivation of P 450 3A4 by EE was irreversible. **Testosterone**, an alternate substrate, was able to protect P 450 3A4 from EE-dependent inactivation. The partition ratio was .apprx.50. The stoichiometry of binding was approx. 1.3 nmol of an EE metabolite bound per nmol of P 450 3A4 inactivated. SDS-polyacrylamide gel electrophoresis **anal.** demonstrated that [3H]EE was irreversibly bound to the P 450 3A4 apoprotein. After extensive dialysis of the [3H]EE inactivated samples, high-pressure **liquid chromatog.** (HPLC) **anal.** demonstrated that the inactivation resulting from EE metabolism led to the destruction of approx. half the heme with the concomitant generation of modified heme and EE-labeled heme fragments and produced covalently radiolabeled P 450 3A4 apoprotein. Electrospray **mass spectrometry** demonstrated that the fraction corresponding to the major radiolabeled product of EE metabolism has a mass (M - H)- of 479 Da. HPLC and gas chromatog.-**mass spectrometry analyses** revealed that EE metabolism by P 450

3A4 generated one major metabolite, 2-hydroxyethynylestradiol, and at least three addnl. metabolites. In conclusion, our results demonstrate that EE is an effective mechanism-based inactivator of P 450 3A4 and that the mechanism of inactivation involves not only heme destruction, but also the irreversible modification of the apoprotein at the active site.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 7 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:96261 HCAPLUS

DOCUMENT NUMBER: 136:195402

TITLE: Enzyme-Assisted Synthesis and Structure Characterization of Glucuronide Conjugates of **Methyltestosterone** (17α-methylandrost-4-en-17β-ol-3-one) and Nandrolone (estr-4-en-17β-ol-3-one) Metabolites

AUTHOR(S): Kuuranne, Tiia; Aitio, Olli; Vahermo, Mikko; Elovaara, Eivor; Kostiainen, Risto

CORPORATE SOURCE: Viikki Drug Discovery Technology Center, University of Helsinki, FIN-00014, Finland

SOURCE: Bioconjugate Chemistry (**2002**), 13(2), 194-199
CODEN: BCCHES; ISSN: 1043-1802

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A new and useful method based on enzyme-assisted synthesis was developed for producing 3α-O-β-D-glucuronide conjugates from synthetic phase I metabolites of **methyltestosterone** and nandrolone. The formed glucuronide conjugates of 17α-methyl-5α-androstane-3α,17β-diol (I), 17α-methyl-5β-androstane-3α,17β-diol (II), 5α-estran-3α-ol-17-one (III), and 5β-estran-3α-ol-17-one (IV) are urinary metabolites, indicating the human misuse of the above-mentioned anabolic androgenic steroids (AAS). The common lack of reference material precludes the use and validation of these biomarkers in human doping control. Liver microsomes from Aroclor 1254-induced rats were used as a highly active source of mammalian UDP-glucuronosyltransferases (UGT, EC 2.4.1.17). After **purification** by protein precipitation, liquid-liquid extraction (dichloromethane), C-18 solid-phase extraction, and lyophilization, the steroid glucuronide structures were characterized by 1H and 13C NMR spectroscopy and tandem **mass spectrometry**. The enzymic method was highly stereoselective, producing a single major conjugate from the parent steroids I-IV. The stereochem. pure steroid glucuronide conjugates were recovered in milligram amts. (1.0-2.8 mg, yield 12-29%), which is sufficient for veterinary and human doping control **analyses**; for pharmaco-, toxico-, and enzyme kinetic studies in the pharmaceutical industry; for clin. labs.; and for forensic medicine. A new sensitive LC-MS method was developed for controlling the product purity in syntheses, as well as for enzyme kinetic characterization of AAS-metabolizing UGT activities in rat liver toward the aglycons I-IV. In this study, the UGT enzymes responsible for the formation of 3α-O-linked glucuronides from the substrates I, II, III, and IV exhibited the specific enzyme activity values: 25, 124, 48, and 212 nmol/mg microsomal protein in a 2-h incubation, resp.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 8 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:506142 HCAPLUS
DOCUMENT NUMBER: 135:235604
TITLE: Investigation of synthetic peptide hormones by **liquid chromatography** coupled to pneumatically assisted electrospray ionization **mass spectrometry**: **analysis** of a synthesis crude of peptide triptorelin
AUTHOR(S): Sanz-Nebot, V.; Toro, I.; Castillo, A.; Barbosa, J.
CORPORATE SOURCE: Departament de Quimica Analitica, Universitat de Barcelona, Barcelona, 08028, Spain
SOURCE: Rapid Communications in Mass Spectrometry (**2001**), 15(13), 1031-1039
CODEN: RCMSEF; ISSN: 0951-4198
PUBLISHER: John Wiley & Sons Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Triptorelin, a synthetic peptide hormone used in the treatment of prostate cancer by reduction in the action of male hormone **testosterone**, was studied here. The synthetic procedure commonly results in unwanted side products that require extensive **purification** and characterization of the synthesis mixture The chromatog. separation of triptorelin from the crude mixture was developed by applying the linear solvation energy relation (LSER) methodol. previously developed, to optimize the composition of the mobile phase to avoid lengthy empirical optimization procedures. Electrospray ionization **mass spectrometry** coupled to **liquid chromatog.** (LC/ES-MS) was used to obtain reliable information on the inevitable side products. The knowledge of the identity of these impurities allows fast optimization of the synthetic procedure and also the therapeutic use of triptorelin peptide hormone.
REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 9 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:439244 HCAPLUS
DOCUMENT NUMBER: 133:173115
TITLE: Consequence of boar edible tissue consumption on urinary profiles of nandrolone metabolites. I. **Mass spectrometric detection** and quantification of 19-norandrosterone and 19-noretiocholanolone in human urine
AUTHOR(S): Le Bizec, Bruno; Gaudin, Isabelle; Monteau, Fabrice; Andre, Francois; Impens, Sandra; De Wasch, Katia; De Brabander, Hubert
CORPORATE SOURCE: LDH-LNR, LDH-LNR, Ecole Nationale Veterinaire, Nantes, F-44087, Fr.
SOURCE: Rapid Communications in Mass Spectrometry (**2000**), 14(12), 1058-1065
CODEN: RCMSEF; ISSN: 0951-4198
PUBLISHER: John Wiley & Sons Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB For the first time in the field of steroid residues in humans, demonstration of 19-norandrosterone (19-NA: 3α-hydroxy-5α-estran-17-one) and 19-noretiocholanolone (19-NE: 3α-hydroxy-5β-estran-17-one) excretion in urine subsequent to boar consumption is reported. Three male volunteers agreed to consume 310 g of tissues from the edible parts (meat, liver, heart and kidney) of a boar. The three individuals delivered urine samples before and during 24 h after meal intake. After deconjugation of phase II metabolites, **purification** and specific derivatization of target metabolites, the urinary exts. were

analyzed by **mass spectrometry.** Identification was carried out using measurements obtained by gas chromatog./high resolution **mass spectrometry** (GC/HRMS) (R = 7000) and **liq**. **chromatog.**/tandem **mass spectrometry** (LC/MS/MS) (pos. electrospray ionization (ESI+)). Quantification was realized using a quadrupole mass filter. 19-NA and 19-NE concns. in urine reached 3.1 to 7.5 μg/L nearby 10 h after boar tissue consumption. Levels returned to endogenous values 24 h after. These two steroids are usually exploited to confirm the exogenous administration of 19-**nortestosterone** (19-NT: 17β-hydroxyestr-4-en-3-one), especially in the antidoping field. The authors have thus proved that eating tissues of non-castrated male pork (in which 17β-nandrolone is present) might induce some false accusations of the abuse of nandrolone in antidoping.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 10 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:95665 HCAPLUS
DOCUMENT NUMBER: 132:260766
TITLE: Quantitation of anabolic hormones and their metabolites in bovine serum and urine by **liquid chromatography**-tandem **mass spectrometry**
AUTHOR(S): Draisci, R.; Palleschi, L.; Ferretti, E.; Lucentini, L.; Cammarata, P.
CORPORATE SOURCE: Istituto Superiore di Sanita, Laboratorio di Medicina Veterinaria, Rome, 00161, Italy
SOURCE: Journal of Chromatography, A (**2000**), 870(1+2), 511-522
CODEN: JCRAEY; ISSN: 0021-9673
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A specific and sensitive method based on tandem **mass spectrometry** with online high-performance **liquid chromatog.** using atmospheric pressure chemical ionization (LC-APCI-MS-MS) for the quantitation of anabolic hormone residues (17β-19-**nortestosterone**, 17β- **testosterone** and progesterone) and their major metabolites (17α-19- **nortestosterone** and 17α- **testosterone**) in bovine serum and urine is reported. [2H2]17β- **Testosterone** was used as internal standard The **analytes** were extracted from urine (following enzymic hydrolysis) and serum samples by liquid-liquid extraction and **purified** by C18 solid-phase extraction Ionization was performed in a heated nebulizer interface operating in the pos. ion mode, where only the protonated mol., [M+H]+, was generated for each **analyte.** This served as precursor ion for collision-induced dissociation and two diagnostic product ions for each **analyte** were identified for the unambiguous hormone confirmation by selected reaction monitoring LC-MS-MS. The overall inter-day precision (relative standard deviation) ranged from 6.37 to 2.10% and from 6.25 to 2.01%, for the bovine serum and urine samples, resp., while the inter-day accuracy (relative error) ranged from -5.90 to -3.18% and from -6.40 to -2.97%, for the bovine serum and urine samples, resp. The limit of quantitation of the method was 0.1 ng/mL for all the hormones in bovine serum and urine. On account of its high sensitivity and specificity the method has been successfully used to confirm illegal hormone administration for regulatory purposes.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 11 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:10200 HCAPLUS
DOCUMENT NUMBER: 132:147703
TITLE: Compared interest between hair **analysis** and urinalysis in doping controls. Results for amphetamines, corticosteroids and anabolic steroids in racing cyclists
AUTHOR(S): Gaillard, Y.; Vayssette, F.; Pepin, G.
CORPORATE SOURCE: Laboratoire TOXLAB, Paris, 75018, Fr.
SOURCE: Forensic Science International (**2000**), 107(1-3), 361-379
CODEN: FSINDR; ISSN: 0379-0738
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Different sensitive methods were developed for testing hair for amphetamines, anabolic steroids and their esters and corticosteroids in doping testing. For amphetamines, 50 mg of hair were digested with 1 M NaOH, extracted with Et acetate, derivatized with TFA and **analyzed** by gas chromatog. pos. chemical-ionization **mass spectrometry.** For corticosteroids, 50 mg of powdered hair were treated with MeOH in an ultrasonic bath and subsequently **purified** using a C18 solid phase extraction column. **Anal.** was realized by high performance **liquid chromatog.** coupled to electrospray-ionization tandem **mass spectrometry.** For anabolic steroids and their esters, 100 mg of powdered hair were treated with methanol in an ultrasonic bath for extraction of esters, then alkaline digested with 1 M NaOH for an optimum recovery of other drugs. The 2 liquid prepns. were subsequently extracted with Et acetate, pooled, then finally highly **purified** using a twin solid phase extraction on aminopropyl and silica cartridges. Residue was derivatized with MSTFA prior to injection. **Anal.** was conducted by gas chromatog. coupled to a triple quadrupole **mass spectrometer.** Thirty cyclists were sampled and tested both in hair and in urine. Amphetamine was **detected** 10 times in hair (out of 19 **analyses**) compared to 6 times in urine (out of 30 **analyses**). Corticosteroids were **detected** 5 times in hair (methylprednisolone 1 case, triamcinolone acetonide 3 cases and hydrocortisone acetate 1 case) in hair (out of 12 **analyses**) compared to 12 times (triamcinolone acetonide 10 cases and betamethasone 2 cases) in urine (out of 30 **analyses**). Anabolic steroids were **detected** twice (nandrolone 1 case, and **testosterone** undecanoate 1 case) in hair (out of 25 **analyses**) compared to none in urine (out of 30 **analyses**).
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 12 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1998:775948 HCAPLUS
DOCUMENT NUMBER: 130:178444
TITLE: Comparison of **purification** procedures for the isolation and **detection** of anabolic residues in feces using gas chromatography-**mass spectrometry**
AUTHOR(S): Hamoir, Thierry; Pottie, Gaspard; Courtheyn, Dirk; De Brabander, Hubert; Delahaut, Philippe; Leyssens, Luc
CORPORATE SOURCE: Institute of Public Health, Brussels, 1050, Belg.
SOURCE: Analyst (Cambridge, United Kingdom) (**1998**), 123(12), 2621-2624
CODEN: ANALAO; ISSN: 0003-2654

PUBLISHER: Royal Society of Chemistry
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Within several regional field labs. and the national reference laboratory a harmonized methodol. for the **anal.** of anabolic residues in fecal samples was developed. The method consists of a liquid-liquid and a solid-phase extraction step, followed by a high-performance **liquid chromatog. purification** step. Using gas chromatog.-**mass spectrometry**, currently illegally used anabolic steroids can be **detected** in feces at the ppb level. Within this context acidification, followed by centrifugation under cooling, allows efficient, practical and rapid defatting of fecal samples. Furthermore, a combination of a silica and an aminopropyl solid-phase extraction column was found to give the best results as regards the sample **purification** process.
REFERENCE COUNT: 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 13 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:501991 HCAPLUS
DOCUMENT NUMBER: 127:172310
TITLE: Confirmation of anabolic hormone residues in bovine blood by micro-HPLC-ion spray-tandem **mass spectrometry**
AUTHOR(S): Draisci, Rosa; Giannetti, Luigi; Lucentini, Luca; Palleschi, Luca; Purificato, Ivana; Moretti, Gabriella
CORPORATE SOURCE: Laboratorio Medicina Veterinaria, Istituto Superiore Sanita, Rome, I-00161, Italy
SOURCE: Journal of High Resolution Chromatography (**1997**), 20(8), 421-426
CODEN: JHRCE7; ISSN: 0935-6304
PUBLISHER: Huethig
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A new method for the specific direct **detection** of natural (progesterone and **testosterone**), and synthetic (17β,19-**nortestosterone** and medroxyprogesterone) hormone residues in bovine serum is reported based on tandem MS with online micro-HPLC (micro-HPLC-MS-MS) using an atmospheric pressure ionization (API) source and an ion spray (IS) interface. 17-**Methyltestosterone** was used as the internal standard **Analytes** were extracted with acetate buffer, **purified** on C18 solid-phase extraction (SPE) cartridge and separated on a reversed-phase C18 micro-HPLC column (300 mm + 1 mm, 5 μm), using MeCN-water (80:20) containing 2 mM NH4OAc as mobile phase, at a flow rate of 10 μL/min. When anabolic hormones were ionized in the IS interface operating in the pos. ion mode, only the protonated mols., [M+H]+, were generated, without evidence of any fragmentation. These served as precursor ions for collision-induced dissociation (CID) and diagnostic daughter ions for each **analyte** were identified to carry out **anal.** by micro-HPLC-MS-MS in the selected reaction monitoring (SRM) mode. For the **analytes** in question, the response of the mass **detector** was related linearly to the quantity of each **analyte** injected between 10 and 300 pg, in the SRM mode. The limit of **detection** (3σ) was 6-7 pg. Recoveries were ≥83% for 17β,19- **nortestosterone**, **testosterone**, and 17-**methyltestosterone**, and 72% for medroxyprogesterone and progesterone. The micro-HPLC-MS-MS method for the **determination** of anabolic hormones in bovine blood required no sample derivatization, minimal sample preparation, and provided a sensitive, selective, and rapid alternative to the existing **purification**, separation,

and **detection** techniques. This very sensitive method was successfully applied to measure bovine serum concns. of natural hormones, such as **testosterone** and progesterone, to confirm any illegal administration of these substances.

L29 ANSWER 14 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:239213 HCAPLUS
DOCUMENT NUMBER: 126:327635
TITLE: Identification of vertebrate type steroid hormones in the shrimp Penaeus japonicus by tandem **mass spectrometry** and sequential product ion scanning
AUTHOR(S): Cardoso, Ana M.; Barros, Cristina M. F.; Correia, A. J. Ferrer; Cardoso, J. M.; Cortez, A.; Carvalho, F.; Baldaia, L.
CORPORATE SOURCE: Dep. Quimica, Univ. Aveiro, Aveiro, 3800, Port.
SOURCE: Journal of the American Society for Mass Spectrometry (**1997**), 8(4), 365-370
CODEN: JAMSEF; ISSN: 1044-0305
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The identification of **testosterone**, pregnenolone, and 17α-hydroxyprogesterone by tandem **mass spectrometry** and of progesterone by sequential product ion scanning in the shrimp gonads of Penaeus japonicus is described. The identification of these substances in biol. samples is usually done by gas chromatog.-**mass spectrometry** and involves several **liquid chromatog. purification** steps followed by derivatization. The utilization of tandem **mass spectrometry** in this **anal.** has simplified considerably the sample pretreatment and provided a very simple method of screening these substances in complex mixts.

L29 ANSWER 15 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:212512 HCAPLUS
DOCUMENT NUMBER: 126:289092
TITLE: Confirming **testosterone** administration by isotope ratio **mass spectrometric analysis** of urinary androstanediols
AUTHOR(S): Shackleton, Cedric H.L.; Phillips, Andy; Chang, Tony; Li, Ye
CORPORATE SOURCE: Children's Hospital Oakland Research Institute, Oakland, CA, 94609, USA
SOURCE: Steroids (**1997**), 62(4), 379-387
CODEN: STEDAM; ISSN: 0039-128X
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A gas chromatog. combustion isotope ratio **mass spectrometric** (GC/C/IRMS) method was used for studying the incorporation of exogenous **testosterone** enanthate into excreted urinary 5α- and 5β-androstane-3α,17β-diols. A multistep but straightforward work-up procedure produced a simple GC chromatogram of urinary steroid acetates composed principally of two androstanediols and pregnanediol. It is anticipated that such a method may form the basis of a doping control test for **testosterone** that could be used as a primary method during major sporting events or alternatively as a verification technique. Urine samples from five individuals were collected before and after administration of

testosterone enanthate (250 mg). The δ13C 0/00 value of androstanediols was around -26 to -28 during the baseline period and decreased to about -29 to -30 in the days following synthetic **testosterone** administration. One of the other major steroids in the chromatogram, pregnanediol, was utilized as the "internal standard," because its δ13C0/00 values did not markedly change following **testosterone** administration, remaining at -25 to -27. In all subjects studied, the δ13C0/00 values for androstanediols were reduced sufficiently over 8 days to confirm administration of synthetic **testosterone.** Although steroids isolated from urine of normal individuals from 12 different countries gave values between -24 and -28, this seemed not to be related to nationality or region. The most likely variable is the proportion of plants with low and high carbon 13 content in the diet. This variable is likely to be more affected by individual food preferences than broad ethnic food divisions. In this paper, we propose a ratio of δ13C0/00 for androstanediols to pregnanediol as a useful discriminant of **testosterone** misuse, a value above 1.1:1.0 being indicative of such misuse. The work-up procedure was designed for batch **anal.** and to use only simple techniques, rather than employ further instrumentation, such as high-performance **liquid chromatog.** (HPLC), in **purifying** steroids for GC/C/IRMS.

REFERENCE COUNT: 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 16 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1997:70604 HCAPLUS
DOCUMENT NUMBER: 126:100340
TITLE: **Detection** of **testosterone** misuse: comparison of two chromatographic-combustion/isotope ratio **mass spectrometric analysis**
AUTHOR(S): Aguilera, R.; Grenot, C.; Casabiance, H.; Hatton, C. K.
CORPORATE SOURCE: Service Central Aanlyse, CNRS, Vernaison, 69390, Fr.
SOURCE: Journal of Chromatography, B: Biomedical Applications (**1996**), 687(1), 43-53
CODEN: JCBBEP; ISSN: 0378-4347
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Two chromatog. methods, reversed-phase **liquid chromatog.** (LC) and immunoaffinity chromatog. (IAC) were compared in the preparation of **purified testosterone** exts. suitable for gas chromatog.-combustion/isotope ratio **mass spectrometry** (GC-C-IRMS) **anal.** We have shown previously that GC-C-IRMS is a promising means of **detection** of **testosterone** misuse in sport. The two clean-up procedures afford sufficient recovery and adequate purity of **testosterone.** LC presents several advantages over IAC: access to other urinary steroids, longer column life, no need for special equipment and no antibody preparation For IAC, the antibodies to **testosterone** must be selected with care for high affinity and low cross-reactivity. Nevertheless, IAC is of some interest in our expts., the recovery is slightly better for low concns. of urinary **testosterone** and IAC does not induce isotopic discrimination even in overloading expts. This is the first report on sample preparation by IAC prior to GC-C-IRMS and carbon isotope ratio values for urinary **epitestosterone.** The carbon isotope ratio test can identify users' urines missed by the **testosterone** to **epitestosterone** ratio (T/E>6) test.

REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 17 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1995:747079 HCAPLUS
DOCUMENT NUMBER: 123:167298
TITLE: A novel nonhepatic hydroxycholesterol 7α-hydroxylase that is markedly stimulated by interleukin-1β. Characterization in the immature rat ovary
AUTHOR(S): Payne, Donna W.; Shackleton, Cedric; Toms, Harold; Ben-Shlomo, Izhar; Kol, Shahar; deMoura, Marcos; Strauss, Jerome F.; Adashi, Eli Y.
CORPORATE SOURCE: Dep. Obstetrics/Gynecol., Univ. Maryland Med. Sch., Baltimore, MD, 21201, USA
SOURCE: Journal of Biological Chemistry (**1995**), 270(32), 18888-96
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB During studies on the regulation of rat ovarian steroidogenic enzymes by interleukin-1β (IL-1β), the authors observed substantial metabolism of 25-hydroxycholesterol to two unusual polar products. This unexpected effect was observed both in isolated granulosa cells and in whole ovarian dispersates and was also induced by tumor necrosis factor α, but not by insulin-like growth factor I or FSH. The effect was dependent on time and the dose of IL-1β and was blocked by an IL-1 receptor antagonist. The formation of the polar metabolites was inhibited by ketoconazole and trilostane, but not by aminoglutethimide. Subsequent **purification** of these novel metabolites and **anal.** by gas chromatog./**mass spectrometry**, NMR, and high performance **liquid chromatog.** revealed them to be related 7α-hydroxylated hydroxycholesterols (cholest-4-ene-7α,25-diol-3-one and cholest-5-ene-3β,7α,25-triol). IL-1β-stimulated ovarian 7α-hydroxylase activity (3-10 pmol/min/mg of cellular protein) was nearly 4-fold that of control levels using 25-hydroxycholesterol as substrate. Activities at or below control levels were observed when IL-1β-treated cell sonicates were boiled or assayed in the presence of NADH (rather than NADPH), indicating that involvement of a nonenzymic process was unlikely. IL-1β-stimulated 7α-hydroxylase activity was inhibited to basal levels by a 10-fold excess of unlabeled 25- or 27-hydroxycholesterol, but not by cholesterol, pregnenolone, progesterone, **testosterone**, or dehydroepiandrosterone, suggesting that ovarian 7α-hydroxylase is specific for hydroxycholesterols. Furthermore, when IL-1β-treated ovarian cultures were incubated with radiolabeled cholesterol or **testosterone**, no 7α-hydroxylated products were observed The authors were also unable to **detect** any mRNA transcripts for liver cholesterol 7α-hydroxylase in IL-1β-stimulated ovarian cultures. This study describes an ovarian hydroxycholesterol 7α-hydroxylase that differs from liver cholesterol 7α-hydroxylase and from other nonhepatic progestin/androgen 7α-hydroxylases. The novel finding of the regulation of a 7α-hydroxylase by IL-1β (and tumor necrosis factor α) suggests a unique role for cytokines in the regulation of cholesterol metabolism in the ovary and possibly other tissues.

L29 ANSWER 18 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1993:225953 HCAPLUS

DOCUMENT NUMBER: 118:225953
TITLE: Metabolism of 17β,19- **nortestosterone** in urine of calves after oral intake and intramuscular administration
AUTHOR(S): Daeseleire, E.; De Guesquiere, A.; Van Peteghem, C.
CORPORATE SOURCE: Lab. Food Anal., Univ. Ghent, Harelbekestraat 72, 9000, Ghent, Belg.
SOURCE: Analytica Chimica Acta (**1993**), 275(1-2), 95-103
CODEN: ACACAM; ISSN: 0003-2670
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The metabolism of 17β,19- **nortestosterone** was investigated in 6 calves. Three calves were injected with Laurabolin and the other 3 were fed 17β,19- **nortestosterone**-containing food. Urine samples were taken before and at regular time intervals after the injection or oral intake. After enzymic hydrolysis, the sample clean-up consisted in solid-phase extraction followed by a **liquid chromatog. purification Detection** was by gas chromatog.-**mass spectrometry.** Urine samples from pregnant and non-pregnant cows and from steers were also collected and **analyzed** in the same way. In addition to estradiol and **testosterone**, 5α-estrane-3β,17α-diol was found in the urine from the pregnant cow and traces of 19-noretiocholanolone was found in the urine from 2 steers. 17β,19- **Nortestosterone** itself or the 17α-epimer were not **detected.** After i.m. injection of Laurabolin the most abundant metabolite in the urine was 17α,19-**nortestosterone**; 5α-estrane-3β-17α-diol and 19-noretiocholanolone were also **detected** in urine from all animals.

L29 ANSWER 19 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:504389 HCAPLUS
DOCUMENT NUMBER: 117:104389
TITLE: Characterization of Met-139 as the photolabeled amino acid residue in the steroid binding site of sex hormone binding globulin using Δ6 derivatives of either **testosterone** or estradiol as unsubstituted photoaffinity labeling reagents
AUTHOR(S): Grenot, Catherine; De Montard, Arnaud; Blachere, Thierry; De Ravel, Marc Rolland; Mappus, Elisabeth; Cuilleron, Claude Y.
CORPORATE SOURCE: Hop. Debrousse, Lyon, 69322, Fr.
SOURCE: Biochemistry (**1992**), 31(33), 7609-21
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Immunopurified** human sex hormone-binding globulin (SHBG) was photoactivated and photolabeled by radioinert and radioactive photoaffinity labeling steroids Δ6- **testosterone** (Δ6-T) and Δ6-estradiol (Δ6-E2). The maximal levels of specific incorporation of these two reagents were 0.50 and 0.33 mol of label/mol of SHBG, resp. Covalently labeled SHBG fractions were citraconylated, reduced, carboxymethylated, and cleaved by trypsin. Separation of tryptic digests by reverse-phase **liquid chromatog.** gave single radioactive peaks at the same retention times with both steroid reagents. However, the two labeled peptidic fractions could be distinguished by capillary electrophoresis and **immunodetection** with anti-steroid antibodies, whereas the covalent attachment of radioactivity was controlled by TLC on silica gel. Edman degradation of the

two labeled peptides showed a single sequence His-Pro-Ile-([3H]X)-Arg corresponding to the pentapeptide His-Pro-Ile-Met-Arg 136-140 of SHBG sequence. The coincidence, in both cases, of the absence of an identifiable amino acid residue and of the elution of the most intense peak of radioactivity at the fourth cycle of Edman degradation suggests that the same Met-139 residue was labeled by Δ6-[1,2-3H]T or by Δ6-[17α-3H]E2. Liquid secondary ion **mass spectrometry** of the two peptides showed [M + H]+ ions at m/z 939.8 or 923.8, corresponding resp. to the addition of Δ6-T or Δ6-E2 to the pentapeptide. The presence of the steroid mol. in the Δ6-[3H]T-pentapeptide conjugate was confirmed by the difference of 2 mass units with the [M + H]+ peak of the Δ6-[4-14C]T-pentapeptide conjugate.

L29 ANSWER 20 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:402637 HCAPLUS
DOCUMENT NUMBER: 115:2637
TITLE: **Detection** of 19-**nortestosterone** and its urinary metabolites in miniature pigs by gas chromatography-**mass spectrometry**
AUTHOR(S): Debruyckere, G.; Van Peteghem, C.
CORPORATE SOURCE: Fac. Pharm. Sci., State Univ. Ghent, Ghent, 9000, Belg.
SOURCE: Journal of Chromatography (**1991**), 564(2), 393-403
CODEN: JOCRAM; ISSN: 0021-9673
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The metabolism of 19-**nortestosterone** was investigated in a miniature noncastrated male pig (boar), in a castrated pig (barrow) and in a female pig (sow). Urine samples were taken before and at regular intervals after the injection of 100 mg of laurabolin (**nortestosterone** laurate). The sample clean-up consists in preliminary solid-phase extraction, followed by high-performance **liquid chromatog. purification** and fractionation. Finally, gas chromatog.-**mass spectrometry** is used to identify the 19-**nortestosterone** metabolites.

L29 ANSWER 21 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:205634 HCAPLUS
DOCUMENT NUMBER: 114:205634
TITLE: Combined high-performance **liquid chromatography** and radioimmunoassay for the screening of 19-**nortestosterone** and **methyltestosterone** residues in meat samples
AUTHOR(S): Daeseleire, E.; De Guesquiere, A.; Van Peteghem, C.
CORPORATE SOURCE: Fac. Pharm. Sci., State Univ. Ghent, Ghent, B-9000, Belg.
SOURCE: Journal of Chromatography (**1991**), 564(2), 445-9
CODEN: JOCRAM; ISSN: 0021-9673
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A procedure is described for the **detection** of 17β-19-**nortestosterone** (17β-19-NT) and 17α-**methyltestosterone** (17α-MT) in muscle tissue by a combination of HPLC (L:Chrospher RP 18 column; MeOH-H2O, 65:35; UV **detection**) and RIA. The steroids were released from the muscle tissue by enzymic digestion, and the exts. were **purified** by solid-phase extraction A com. meat sample, which was proved to contain

17β-19-NT and 17α-MT residues by gas chromatog.- **mass spectrometry**, and blank meat samples obtained from non-treated exptl. animals were **purified**, and the exts. injected into the **liquid chromatograph.** With an automatic fraction collelctor, fifteen fractions of 1 mL each were obtained, which after evaporation were subjected to a RIA for the steroid. None of the fractions from the blank meat samples contained any substance that interfered with the immunochem. **detection** system. Good qual. agreement between the two sets of results was obtained. Although the sample preparation step is labor-intensive, the method can be successfully applied as a reliable confirmation method for pos. RIA screening results in circumstances where no gas chromatog.-**mass spectrometry** facilities are available.

L29 ANSWER 22 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1984:17943 HCAPLUS
DOCUMENT NUMBER: 100:17943
TITLE: Steroids in porcine follicular fluid: **analysis** by HPLC, capillary GC and capillary GC/MS after **purification** on SEP-PAK C18 and ion exchange chromatography
AUTHOR(S): Khalil, M. Wahid; Lawson, V.
CORPORATE SOURCE: Group Reprod. Biol., MRC, London, ON, N6A 5A5, Can.
SOURCE: Steroids (**1983**), 41(4), 549-67
CODEN: STEDAM; ISSN: 0039-128X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Testosterone** [**58-22-0**], progesterone [57-83-0] and androstenedione [63-05-8] were the major steroids **detected** in porcine follicular fluid by high-pressure **liquid chromatog**. (HPLC) monitored at 254 nm, although 17-hydroxy [68-96-2], 20α-dihydro- [145-14-2] and 20β-dihydroprogesterone [145-15-3] were also present. Pregnenolone [145-13-1], pregnanediol [80-92-2], dehydroepiandrosterone [53-43-0], 17-hydroxypregnenolone [387-79-1] and androsterone [53-41-8] were **detected** by capillary gas chromatog. (GC) or capillary GC/**mass spectroscopy** (MS) as their O-methyloxime trimethylsilyl ether derivs. after **purification** on SEP-PAK C18 and ion-exchange chromatog. Further confirmation of structure was provided by complete **mass spectral** data or by selective ion monitoring.

L29 ANSWER 23 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1972:511872 HCAPLUS
DOCUMENT NUMBER: 77:111872
TITLE: Occurrence of **testosterone** and 5α-**dihydrotestosterone** in the submaxillary salivary gland of the boar
AUTHOR(S): Booth, W. D.
CORPORATE SOURCE: Agric. Res. Counc. Unit. Reproductive Physiol. Biochem., Univ. Cambridge, Cambridge, UK
SOURCE: Journal of Endocrinology (**1972**), 55(1), 119-25
CODEN: JOENAK; ISSN: 0022-0795
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Testosterone** and 5α- **dihydrotestosterone** were extracted from the submaxillary salivary glands of boars at different ages, but were not **detected** in these glands of the female pig. After **purification** by thin-layer and paper chromatog. the steroids were identified by gas-**liquid chromatog.** and combined gas-

liquid chromatog.-mass spectrometry.
In the submaxillary gland of the mature boar, a high concentration of androgen (>1 μg/100 g) was found, and the concentration of 5α-**dihydrotestosterone** was 2-4 times higher than **testosterone**. Immediate steroid precursors of **testosterone** which are found in boar testis were not isolated. The isolation of the 2 potent androgens, and the known occurrence of 16-unsatd. C19 steroids in the boar submaxillary gland, shows that there is a biochem. sexual dimorphism in this gland of the pig, which is primarily under the influence of testicular hormones.

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=> d que stat l31
L23           1 SEA FILE=REGISTRY ABB=ON  TESTOSTERONE/CN
L24         970 SEA FILE=HCAPLUS ABB=ON  (L23 OR ?TESTOSTERON?) AND ?MASS?(W)?S
                PECT?
L25         822 SEA FILE=HCAPLUS ABB=ON  L24 AND (?DETECT? OR ?DETERMIN? OR
                ?ANAL?)
L27         177 SEA FILE=HCAPLUS ABB=ON  L25 AND LIQ?(W)?CHROMATOG?
L28          23 SEA FILE=HCAPLUS ABB=ON  L27 AND ?PURIF?
L29          23 SEA FILE=HCAPLUS ABB=ON  L28 AND (PRD<20031202 OR PD<20031202)

L30          62 SEA L29
L31          49 DUP REMOV L30 (13 DUPLICATES REMOVED)

L31  ANSWER 1 OF 49  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
ACCESSION NUMBER:   2003446300  EMBASE
TITLE:              Neurosteroids in Rat Brain: Extraction, Isolation, and
                    Analysis by Nanoscale Liquid
                    Chromatography-Electrospray Mass
                    Spectrometry.
AUTHOR:             Liu S.; Sjovall J.; Griffiths W.J.
CORPORATE SOURCE:   W.J. Griffiths, Dept. of Med. Biochem. and Biophys.,
                    Karolinska Institutet, SE-171 77 Stockholm, Sweden.
                    william.griffiths@ulsop.ac.uk
SOURCE:             Analytical Chemistry, (1 Nov 2003) 75/21
                    (5835-5846).
                    Refs: 36
                    ISSN: 0003-2700  CODEN: ANCHAM
COUNTRY:            United States
DOCUMENT TYPE:      Journal; Article
FILE SEGMENT:       003     Endocrinology
                    008     Neurology and Neurosurgery
                    029     Clinical Biochemistry
LANGUAGE:           English
SUMMARY LANGUAGE:   English
AB   A method designed for the analysis of sulfated neurosteroids and
     unconjugated ketonic neurosteroids in rat brain using nanoscale
     liquid chromatography-electrospray (nano-LC-ES)
     mass spectrometry is described. Neurosteroids in rat
     brain tissue were extracted, purified, and separated into two
     groups, neutral unconjugated steroids and steroid sulfates, by employing
     solid-phase partition, cation- and anion-exchange chromatography. The
     steroid sulfate fraction was analyzed by nano-LC-ES mass
     spectrometry. Contrary to expectations, the sulfates of
     pregnenolone and dehydroepiandrosterone (DHEA) were not detected
     . Internal standards, including pregnenolone sulfate, were recovered and
     the detection limit of the method was 0.3 ng/g of wet brain.
     Cholesterol sulfate was detected at a level of 1.2 μg/g of
     wet brain. The neutral unconjugated steroid fraction was derivatized with
     hydroxylamine hydrochloride to convert oxosteroids into their oximes. The
     oximes were isolated using cation-exchange chromatography and were
     analyzed by nano-LC-ES tandem mass spectrometry
     . The analyses of the neutral unconjugated steroid fraction
     confirmed the presence in rat brain of pregnenolone, pregnanolone isomers,
     progesterone, testosterone, and DHEA, which were characterized
     by their retention times, the mass of the protonated molecules, and
     characteristic fragment ions. The levels were estimated by addition of
     [3,4-(13)C (2)]-progesterone as an internal standard and found to be in a
     range of 0.04-20 ng/g.
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L31 ANSWER 2 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 1

ACCESSION NUMBER: 2003:330375 BIOSIS
DOCUMENT NUMBER: PREV200300330375
TITLE: Confirmatory **analysis** of 17beta-boldenone, 17alpha-boldenone and androsta-1,4-diene-3,17-dione in bovine urine by **liquid chromatography** -tandem **mass spectrometry.**
AUTHOR(S): Draisci, Rosa [Reprint Author]; Palleschi, Luca; Ferretti, Emanuele; Lucentini, Luca; Delli Quadri, Fernanda
CORPORATE SOURCE: Laboratorio di Medicina Veterinaria, Istituto Superiore di Sanita, V.le Regina Elena, 299, 00161, Rome, Italy draisci@iss.it
SOURCE: Journal of Chromatography B, (**15 June 2003**) Vol. 789, No. 2, pp. 219-226. print. ISSN: 1570-0232 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Jul 2003 Last Updated on STN: 22 Aug 2003

AB A sensitive and selective **liquid chromatography**-tandem **mass spectrometry** (LC-MS-MS) method for confirmatory **analysis** of 17beta-boldenone (17beta-BOL), 17alpha-boldenone (17alpha-BOL) and androsta-1,4-diene-3,17-dione (ADD) in bovine urine was developed. (2H2)17beta-**Testosterone** (17beta-T-d2) was used as the internal standard. Sample preparation involved enzymatic hydrolysis and **purification** on a C18 solid-phase extraction column. Chromatographic separation of the **analytes** was obtained using an RP-C18 HPLC column. LC-MS-MS **detection** was carried out with an atmospheric pressure chemical ionisation (APCI) source equipped with a heated nebulizer (HN) interface operating in the positive ion mode. For unambiguous hormone confirmation, three **analyte** precursor-product ion combinations were monitored during multiple-reaction monitoring (MRM) LC-MS-MS **analysis.** Overall recovery (%), repeatability (relative standard deviations, RSD, %) and within-laboratory reproducibility (RSD, %) ranged from 92.2 to 97.7%, from 6.50 to 2.94% and from 13.50 to 5.04%, respectively, for all **analytes.** The limit of quantification in bovine urine was 0.20 ng ml-1 for 17beta-BOL and ADD and 0.50 ng ml-1 for 17alpha-BOL. The validated method was successfully applied for **determination** of 17beta-BOL, 17alpha-BOL and ADD in a large number of bovine urine samples collected within the national Official Residue Control Program.

L31 ANSWER 3 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 2

ACCESSION NUMBER: 2003:288896 BIOSIS
DOCUMENT NUMBER: PREV200300288896
TITLE: Screening for gestagens in kidney fat using accelerated solvent extraction and **liquid chromatography** electrospray tandem **mass spectrometry.**
AUTHOR(S): Hooijerink, H. [Reprint Author]; van Bennekom, E. O.; Nielen, M. W. F.
CORPORATE SOURCE: Rijks-kwaliteitsinstituut voor land-en tuinbouwproducten, State Institute for Quality Control of Agricultural Products, Bornsesteeg 45, 6700 AE, P.O. Box 230, Wageningen, Netherlands h.hooijerink@rikilt.wag-ur.nl
SOURCE: Analytica Chimica Acta, (**25 April 2003**) Vol. 483,

No. 1-2, pp. 51-59. print.
ISSN: 0003-2670 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 19 Jun 2003
Last Updated on STN: 1 Aug 2003

AB A screening method has been developed for the **determination** of various anabolic steroids in kidney fat. Fat samples are extracted and steroids are trapped "on-line" during accelerated solvent extraction (ASE). Following this initial extraction samples are further **purified** with C18 solid-phase extraction (SPE). Compounds were finally **analyzed** by **liquid chromatography** (LC) coupled with tandem **mass spectrometry** (MS). The method was validated using blank kidney fat and fat samples fortified at 2 ng g-1. The **detection** capability (CCbeta) was <2 ng g-1 and the decision limit (CCalpha) varied from 0.3 to 0.9 ng g-1. Recoveries were in the range 17-58%, the accuracy using isotopically-labeled internal standards varied from 100 to 135%, and the method was successfully applied to screening of the gestagens flurogestone acetate (FGA), delmadinone acetate (DMA), megestrol acetate (MEGA), chloromadinone acetate (CMA), melengestrol acetate (MELA) and medroxyprogesterone acetate (MPA), and the androgen **chlorotestosterone** acetate (CTA) in kidney fat.

L31 ANSWER 4 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 2002:609959 BIOSIS
DOCUMENT NUMBER: PREV200200609959
TITLE: Development of an **analytical** method to **analyse** steroids in mussels.
AUTHOR(S): Budzinski, H. [Reprint author]; Togola, A. [Reprint author]; Devier, M. H. [Reprint author]; Augagneur, S. [Reprint author]; Narbonne, J. F. [Reprint author]; Garrigues, P. [Reprint author]
CORPORATE SOURCE: LPTC-UMR 5472 CNRS, University of Bordeaux I, Bordeaux, France
SOURCE: Marine Environmental Research, (**September-December, 2002**) Vol. 54, No. 3-5, pp. 749-750. print.
Meeting Info.: Eleventh International Symposium on Pollutant Responses in Marine Organisms (PRIMO 11). Plymouth, UK. July 10-13, 2001.
CODEN: MERSDW. ISSN: 0141-1136.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Nov 2002
Last Updated on STN: 27 Nov 2002

L31 ANSWER 5 OF 49 JICST-EPlus COPYRIGHT 2005 JST on STN
ACCESSION NUMBER: 1020456084 JICST-EPlus
TITLE: The Isolation, Identification and **Determination** of Dehydrotumulosic Acid in Poria cocos.
AUTHOR: SONG Z; BI K; LUO X
CHAN K
CORPORATE SOURCE: Shenyang Pharmaceutical Univ., Shenyang, Chn
Hong Kong Baptist Univ., Hkg
SOURCE: Anal Sci, (2002) vol. 18, no. 5, pp. 529-531. Journal Code: G0673B (Fig. 2, Tbl. 2, Ref. 4)
CODEN: ANSCEN; ISSN: 0910-6340
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article

LANGUAGE: English
STATUS: New
AB Poria cocos (Fuling), a popular Chinese medicinal (CM) herb of fungal origin, has been included in many combinations with other CM herbs for its traditionally claimed activities of inducing diuresis, excreting dampness, invigorating the spleen and tranquilizing the mind and its modern pharmacological use of modulating the immune system of the body. Dehydrotumulosic acid, one of the effective constituents of Fuling, was isolated from the chloroform-soluble material of ethanol extract of the fungus. After further **purification** by a high-performance **liquid chromatographic** method on a C18 column, the **purified** constituent was identified using modern **analytical** techniques, such as UV, 13C-NMR and EI-MS. A reversed-phase high-performance **liquid chromatographic** method has been developed for the **determination** of dehydrotumulosic acid in Poria cocos. The **determination** can be accomplished in less than 50 min using methanol-acetonitrile-2% glacial acetic acid as the mobile phase at a flow rate of 1.0 mL/min, with a UV **detector** setting at 242 nm and **testosterone** propionate used as an internal standard. This assay for dehydrotumulosic acid is simple, rapid and with good reproducibility. (author abst.)

L31 ANSWER 6 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 2002:248650 BIOSIS
DOCUMENT NUMBER: PREV200200248650
TITLE: Enzyme-assisted synthesis and structure characterization of glucuronide conjugates of **methyltestosterone** (17alpha-methylandrost-4-en-17beta-ol-3-one) and nandrolone (estr-4-en-17beta-ol-3-one) metabolites.
AUTHOR(S): Kuuranne, Tiia; Aitio, Olli; Vahermo, Mikko; Elovaara, Eivor; Kostiainen, Risto [Reprint author]
CORPORATE SOURCE: Viikki Drug Discovery Technology Center, University of Helsinki, FIN-00014, Helsinki, Finland
risto.kostiainen@helsinki.fi
SOURCE: Bioconjugate Chemistry, (**March-April, 2002**) Vol. 13, No. 2, pp. 194-199. print.
CODEN: BCCHES. ISSN: 1043-1802.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 17 Apr 2002
Last Updated on STN: 17 Apr 2002
AB A new and useful method based on enzyme-assisted synthesis was developed for producing 3alpha-O-beta-D-glucuronide conjugates from synthetic phase I metabolites of **methyltestosterone** and nandrolone. The formed glucuronide conjugates of 17alpha-methyl-5alpha-androstane-3alpha,17beta-diol (I), 17alpha-methyl-5beta-androstane-3alpha,17beta-diol (II), 5alpha-estran-3alpha-ol-17-one (III), and 5beta-estran-3alpha-ol-17-one (IV) are urinary metabolites, indicating the human misuse of the above-mentioned anabolic androgenic steroids (AAS). The common lack of reference material precludes the use and validation of these biomarkers in human doping control. Liver microsomes from Aroclor 1254-induced rats were used as a highly active source of mammalian UDP-glucuronosyltransferases (UGT, EC 2.4.1.17). After **purification** by protein precipitation, liquid-liquid extraction (dichloromethane), C-18 solid-phase extraction, and lyophilization, the steroid glucuronide structures were characterized by 1H and 13C NMR spectroscopy and tandem **mass spectrometry.** The enzymatic method was highly stereoselective, producing a single major conjugate from the parent steroids I-IV. The stereochemically pure steroid glucuronide conjugates

were recovered in milligram amounts (1.0-2.8 mg, yield 12-29%), which is sufficient for veterinary and human doping control **analyses**; for pharmaco-, toxico-, and enzyme kinetic studies in the pharmaceutical industry; for clinical laboratories; and for forensic medicine. A new sensitive LC-MS method was developed for controlling the product purity in syntheses, as well as for enzyme kinetic characterization of AAS-metabolizing UGT activities in rat liver toward the aglycones I-IV. In this study, the UGT enzymes responsible for the formation of 3alpha-O-linked glucuronides from the substrates I, II, III, and IV exhibited the specific enzyme activity values: 25, 124, 48, and 212 nmol/mg microsomal protein in a 2-h incubation, respectively.

L31 ANSWER 7 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2002:339209 BIOSIS
DOCUMENT NUMBER: PREV200200339209
TITLE: **Testosterone** metabolism in the estuarine Mysid Neomysis integer (Crustacea; Mysidacea): Identification of **testosterone** metabolites and endogenous vertebrate-type steroids.
AUTHOR(S): Verslycke, Tim [Reprint author]; De Wasch, Katia; De Brabander, Hubert F.; Janssen, Colin R.
CORPORATE SOURCE: Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, J. Plateaustraat 22, B-9000, Ghent, Belgium
tim.verslycke@rug.ac.be
SOURCE: General and Comparative Endocrinology, (**April, 2002**) Vol. 126, No. 2, pp. 190-199. print.
CODEN: GCENA5. ISSN: 0016-6480.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Jun 2002
Last Updated on STN: 12 Jun 2002

AB **Testosterone** metabolism by Neomysis integer (Crustacea; Mysidacea) was assessed to obtain initial data on its metabolic capacity. N. integer were exposed to both **testosterone** and (14C) **testosterone.** Identification of **testosterone** metabolites and endogenous steroids was performed using thin-layer chromatography and **liquid chromatography** with multiple **mass spectrometry.** Endogenous production of **testosterone** in mysids was **detected** for the first time. N. integer were exposed to **testosterone** and metabolized administered **testosterone** extensively. At least 11 polar **testosterone** metabolites (Rf,metabolite < Rf,**testosterone**), androstenedione, **dihydrotestosterone**, and **testosterone** were produced in vivo by N. integer. A sex-specific **testosterone** metabolism was also observed, although this observation requires further confirmation. The anabolic steroid beta-boldenone was also identified for the first time in invertebrates. The metabolic pathway leading to the formation of beta-boldenone remains unknown, since the steroidal precursor androstadienedione could not be **detected.** These results reveal interesting similarities in enzyme systems in invertebrate and vertebrate species. Alterations in steroid hormone metabolism may be used as a new biomarker for the effects of endocrine disruptors in invertebrates.

L31 ANSWER 8 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 3

ACCESSION NUMBER: 2002:261123 BIOSIS
DOCUMENT NUMBER: PREV200200261123

TITLE: Mechanism-based inactivation of cytochrome P450 3A4 by 17alpha-ethynylestradiol: Evidence for heme destruction and covalent binding to protein.
AUTHOR(S): Lin, Hsia-lien; Kent, Ute M.; Hollenberg, Paul F. [Reprint author]
CORPORATE SOURCE: Department of Pharmacology, University of Michigan School of Medicine, 1150 West Medical Center Drive, 2301 MSRB III, Ann Arbor, MI, 48109-0632, USA
phollen@umich.edu
SOURCE: Journal of Pharmacology and Experimental Therapeutics, (**April, 2002**) Vol. 301, No. 1, pp. 160-167. print.
CODEN: JPETAB. ISSN: 0022-3565.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Apr 2002
Last Updated on STN: 24 Apr 2002

AB 17alpha-Ethynylestradiol (EE), a major constituent of many oral contraceptives, inactivated the **testosterone** 6beta-hydroxylation activity of **purified** P450 3A4 reconstituted with phospholipid and NADPH-cytochrome P450 reductase in a mechanism-based manner. The inactivation of P450 3A4 followed pseudo first order kinetics and was dependent on NADPH. The values for the Kl and kinact were 18 muM and 0.04 min-1, respectively, and the t1/2 was 16 min. Incubation of 50 muM EE with P450 3A4 at 37degreeC for 30 min resulted in a 67% loss of **testosterone** 6beta-hydroxylation activity accompanied by a 35% loss of the spectral absorbance of the native protein at 415 nm and a 70% loss of the spectrally **detectable** P450-CO complex. The inactivation of P450 3A4 by EE was irreversible. **Testosterone**, an alternate substrate, was able to protect P450 3A4 from EE-dependent inactivation. The partition ratio was apprx50. The stoichiometry of binding was approximately 1.3 nmol of an EE metabolite bound per nmol of P450 3A4 inactivated. SDS-polyacrylamide gel electrophoresis **analysis** demonstrated that (3H)EE was irreversibly bound to the P450 3A4 apoprotein. After extensive dialysis of the (3H)EE inactivated samples, high-pressure **liquid chromatography** (HPLC) **analysis** demonstrated that the inactivation resulting from EE metabolism led to the destruction of approximately half the heme with the concomitant generation of modified heme and EE-labeled heme fragments and produced covalently radiolabeled P450 3A4 apoprotein. Electrospray **mass spectrometry** demonstrated that the fraction corresponding to the major radiolabeled product of EE metabolism has a mass (M-H)- of 479 Da. HPLC and gas chromatography-**mass spectometry analyses** revealed that EE metabolism by P450 3A4 generated one major metabolite, 2-hydroxyethynylestradiol, and at least three additional metabolites. In conclusion, our results demonstrate that EE is an effective mechanism-based inactivator of P450 3A4 and that the mechanism of inactivation involves not only heme destruction, but also the irreversible modification of the apoprotein at the active site.

L31 ANSWER 9 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 2002:555853 BIOSIS
DOCUMENT NUMBER: PREV200200555853
TITLE: Identification of dehydroepiandrosterone metabolites formed from human prostate homogenate using **liquid chromatography-mass spectrometry** and gas chromatography-**mass spectrometry**
AUTHOR(S): Mitamura, Kuniko; Nakagawa, Takashi; Shimada, Kazutake

[Reprint author]; Namiki, Mikio; Koh, Eitetsu; Mizokami, Atsushi; Honma, Seijiro
CORPORATE SOURCE: Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa, 920-0934, Japan shimada@dbs.p.kanazawa-u.ac.jp
SOURCE: Journal of Chromatography A, (**28 June, 2002**) Vol. 961, No. 1, pp. 97-105. print.
CODEN: JOCRAM. ISSN: 0021-9673.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Oct 2002
Last Updated on STN: 30 Oct 2002

AB The identification of the in vitro metabolites of dehydroepiandrosterone formed from human prostate homogenate was investigated by hyphenated techniques using the stable-isotope dilution method. A mixture of dehydroepiandrosterone and (2H4)dehydroepiandrosterone was incubated with hypertrophied human prostate tissue homogenate in the presence of NAD, NADH and NADPH. The metabolites were extracted with AcOEt-hexane, **purified** by solid-phase extraction, and then **analyzed** by LC-atmospheric pressure chemical ionization MS and/or GC-MS. Androst-5-ene-3beta, 17beta-diol (major product), androst-4-ene-3,17-dione, **testosterone**, 5alpha-**dihydrotestosterone**, androsterone, and 7alpha-hydroxydehydroepiandrosterone were identified in comparison with authentic samples based on their chromatographic behavior and **mass spectra.**

L31 ANSWER 10 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:378868 BIOSIS
DOCUMENT NUMBER: PREV200100378868
TITLE: Investigation of synthetic peptide hormones by **liquid chromatography** coupled to pneumatically assisted electrospray ionization **mass spectrometry**: **Analysis** of a synthesis crude of peptide triptorelin.
AUTHOR(S): Sanz-Nebot, V. [Reprint author]; Toro, I.; Castillo, A.; Barbosa, J.
CORPORATE SOURCE: Departament de Quimica Analitica, Universitat de Barcelona, Diagonal 647, 08028, Barcelona, Spain nebot@zeus.qui.ub.es
SOURCE: Rapid Communications in Mass Spectrometry, (**2001**) Vol. 15, No. 13, pp. 1031-1039. print.
CODEN: RCMSEF. ISSN: 0951-4198.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Aug 2001
Last Updated on STN: 19 Feb 2002

AB Triptorelin, a synthetic peptide hormone used in the treatment of prostate cancer by means of reduction in the action of male hormone **testosterone**, is studied here. The synthetic procedure commonly results in unwanted side products that require extensive **purification** and characterization of the synthesis mixture. The chromatographic separation of triptorelin from the crude mixture was developed by applying the linear solvation energy relationship (LSER) methodology previously developed, to optimize the composition of the mobile phase in order to avoid lengthy empirical optimization procedures. Electrospray ionization **mass spectrometry** coupled to **liquid chromatography** (LC/ES-MS) was used to obtain reliable information on the inevitable side products. The knowledge of the identity of these impurities allows fast optimization of the synthetic

procedure and also the therapeutic use of triptorelin peptide hormone.

L31 ANSWER 11 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2001:427049 BIOSIS
DOCUMENT NUMBER: PREV200100427049
TITLE: **Liquid chromatography** tandem **mass spectrometry** applied to the **analysis** of natural and synthetic steroids in environmental waters.
AUTHOR(S): Lagana, A. [Reprint author]; Fago, G.; Marino, A.; Santarelli, D.
CORPORATE SOURCE: Department of Chemistry, "La Sapienza" University, P.le Aldo Moro 5, 00185, Roma, Italy
aldo.lagana@uniroma1.it
SOURCE: Analytical Letters, (**April, 2001**) Vol. 34, No. 6, pp. 913-926. print.
CODEN: ANALBP. ISSN: 0003-2719.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Sep 2001
Last Updated on STN: 22 Feb 2002

AB A multiresidue **analytical** method for the **determination** of the most common and biologically active natural and synthetic steroids (four estrogens: estriol, 17beta-estradiol, 17alpha-ethynylestradiol, estrone; one progestagen: progesterone and six androgens: trenbolone, boldenone, nandrolone, **testosterone**, 17alpha-**methyltestosterone**, stanozolol) in environmental waters was developed. The **analytes** were isolated from water samples by solid phase extraction (SPE) utilizing a graphitized carbon black adsorbent (Carbograph-1). The final samples were **analyzed** by reversed-phase high performance **liquid chromatography** with tandem **mass spectrometry** using atmospheric pressure chemical ionization (LC-APCI-MS-MS). Ionization was performed in a heated nebulizer (HN) interface operating in the positive ion mode. The protonated ions (M+H)+ and the dehydrated ions (M+H-H2O)+ (for estriol, 17alpha-estradiol, 17beta-estradiol, and 17alpha-ethynylestradiol) were used as precursor ion for collision-induced dissociation (CID), and two diagnostic product ions for each **analyte** were identified for the unambiguous steroid confirmation by multiple reaction monitoring (MRM) mode. Method performance, for this **analytical** procedure, was validated by **analyzing** groundwater and river water samples fortified at level of 20 ng/L. The average recovery for each **analyte** exceeded 82%. Good method precision was demonstrated with percent relative standard deviation of less than 7.2% for all **analytes.**

L31 ANSWER 12 OF 49 EMBASE on STN

ACCESSION NUMBER: 2001350192 EMBASE
TITLE: Identification of a novel phosphorylation site in human androgen receptor by **mass spectrometry.**
AUTHOR: Zhu Z.; Becklin R.R.; Desiderio D.M.; Dalton J.T.
CORPORATE SOURCE: J.T. Dalton, 242 L. M. Parks Hall, Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, United States.
dalton.1@osu.edu
SOURCE: Biochemical and Biophysical Research Communications, (**2001**) 284/3 (836-844).
Refs: 29
ISSN: 0006-291X CODEN: BBRCA

COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB An N-terminal hexahistidine-tagged full-length human androgen receptor protein (His(6)-hAR) was overexpressed and **purified** to apparent homogeneity in the presence of **dihydrotestosterone** (DHT) in our previous studies. In-gel trypsin digestion of the **purified** DHT-bound His(6)-hAR, and tryptic peptide mapping using matrix-assisted laser desorption/ionization time-of-flight **mass spectrometry** (MALDI/TOF-MS), **detected** a total of 17 peptides (21% coverage of hAR) with 9 peptides originating from the ligand-binding domain (LBD, 31% coverage of LBD). Amino acid sequencing **analysis** of the tryptic peptides from a separate in-gel digestion of the His(6)-hAR, using HPLC-coupled electrospray ionization ion trap **mass spectrometry** (LC/ESI-ITMS and MS/MS), unambiguously confirmed 21 peptides with 19% coverage of the hAR, of which 11 peptides originated from the LBD (35% coverage of LBD). These 21 peptides included 11 out of the 17 peptides **detected** by MALDI/TOF-MS. In addition, a novel serine phosphorylation site (Ser(308)) within the N-terminal transactivation domain of hAR was identified. .COPYRGT. 2001 Academic Press.

L31 ANSWER 13 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2001:150683 BIOSIS
DOCUMENT NUMBER: PREV200100150683
TITLE: The quantification of endogenous steroids in bovine aqueous humour and vitreous humour using isotope dilution GC-NCI-MS.
AUTHOR(S): Iqbal, Zafar [Reprint author]; Midgely, John M.; Watson, David G.
CORPORATE SOURCE: Department of Pharmacy, University of Peshawar, Peshawar, 25120, Pakistan
zafariqbal_735@yahoo.com
SOURCE: Journal of Pharmaceutical and Biomedical Analysis, (**February, 2001**) Vol. 24, No. 4, pp. 535-543. print.
CODEN: JPBADA. ISSN: 0731-7085.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Mar 2001
Last Updated on STN: 15 Feb 2002

AB Pentafluorobenzyloxime-trimethylsilyl derivatives of androgens, progestogens and corticosteroids were prepared and used for the **analysis** of these steroids in bovine aqueous humour and vitreous humour by GC-MS method. Appropriate deuteriated isotopomers of the parent steroids were labelled with deuterium via simple synthetic procedure and used as internal standards. The concentration (ng ml-1, +- S.E.M.) of these steroids in bovine aqueous humour and vitreous humour were found to be as follow: (1) aqueous humour (n = 17): hydrocortisone (n = 17; 2.40 +- 0.54), progesterone (n = 15; 0.06 +- 0.01), 4-androstene-3,17-dione (n = 8; 0.15 +- 0.07) and **testosterone** (n = 4; 0.14 +- 0.04); and (2) bovine vitreous humour (n = 19): hydrocortisone (n = 19; 1.78 +- 0.25), progesterone (n = 18; 0.09 +- 0.01), 4-androstene-3,17-dione (n = 19; 0.11 +- 0.02), 11-deoxycorticosterone (n = 12; 29.27 +- 6.42), 17alpha-hydroxyprogesterone (n = 6; 5.55 +- 3.12). The concentration of corticosterone, 11-deoxycorticosterone and 17alpha-hydroxyprogesterone and **testosterone** and corticosterone were below the limit of **detection** in aqueous humour and vitreous humour, respectively.

L31 ANSWER 14 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:364360 BIOSIS
DOCUMENT NUMBER: PREV200000364360
TITLE: Consequence of boar edible tissue consumption of urinary profiles of nandrolone metabolites. I. **Mass spectrometric detection** and quantification of 19-norandrosterone and 19-noretiocholanolone in human urine.
AUTHOR(S): Le Bizec, Bruno [Reprint author]; Gaudin, Isabelle; Monteau, Fabrice; Andre, Francois; Impens, Sandra; De Wasch, Katia; De Brabander, Hubert
CORPORATE SOURCE: LDH-LNR, Ecole Nationale Veterinaire, F-44087, Nantes Cedex, 03, France
SOURCE: Rapid Communications in Mass Spectrometry, (**2000**) Vol. 14, No. 12, pp. 1058-1065. print.
CODEN: RCMSEF. ISSN: 0951-4198.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Aug 2000
Last Updated on STN: 8 Jan 2002

AB For the first time in the field of steroid residues in humans, demonstration of 19-norandrosterone (19-NA: 3alpha-hydroxy-5alpha-estran-17-one) and 19-noretiocholanolone (19-NE: 3alpha-hydroxy-5beta-estran-17-one) excretion in the urine subsequent to boar consumption is reported. Three male volunteers agreed to consume 310 g of tissues from the edible parts (meat, liver, heart and kidney) of a boar. The three individuals delivered urine samples before and during 24 h after meal intake. After deconjugation of phase II metabolites, **purification** and specific derivatisation of target metabolites, the urinary extracts were **analysed** by **mass spectrometry.** Identification was carried out using measurements obtained by gas chromatography/high resolution **mass spectrometry** (GC/HRMS) (R=7000) and **liquid chromatography**/tandem **mass spectrometry** (LC/MS/MS) (positive electrospray ionisation (ESI+)). Quantification was realised using a quadrupole mass filter. 19-NA and 19-NE concentrations in urine reached 3.1 to 7.5 mug/L nearby 10 hours after boar tissue consumption. Levels returned to endogenous values 24 hours after. These two steroids are usually exploited to confirm the exogenous administration of 19-nortesterone (19-NT: 17beta-hydroxyestr-4-en-3-one), especially in the antidoping field. We have thus proved that eating tissues of non-castrated male pork (in which 17beta-nandrolone is present) might induce some false accusations of the abuse of nandrolone in antidoping.

L31 ANSWER 15 OF 49 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2001126738 EMBASE
TITLE: Identification of the lipophilic factor produced by macrophages that stimulates steroidogenesis.
AUTHOR: Nes W.D.; Lukyanenko Y.O.; Zhong Hua Jia; Quideau S.; Howald W.N.; Pratum T.K.; West R.R.; Hutson J.C.
CORPORATE SOURCE: Dr. J.C. Hutson, Department of Cell Biology, Texas Tech Univ. Health Sci. Center, Lubbock, TX 79430, United States. jim.hutson@ttmc.ttuhsc.edu
SOURCE: Endocrinology, (**2000**) 141/3 (953-958).
Refs: 33
ISSN: 0013-7227 CODEN: ENDOAO
COUNTRY: United States

DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 003 Endocrinology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Macrophages are known to release a lipophilic factor that stimulates **testosterone** production by Leydig cells. This macrophage-derived factor (MDF) is thought to be physiologically relevant, because removal of macrophages from the testis results in altered **testosterone** secretion and reduced fertility. The purpose of the present study was to **purify** this factor, elucidate its chemical structure, and **determine** whether it is both present in the testis and acts when injected intratesticularly. Culture media from testicular and peritoneal macrophages were extracted with ether, and the organic phase was sequentially **purified** on C(18), silica, and cyano-HPLC columns. MDF was **detected** using a rat Leydig cell bioassay, with **testosterone** secretion being the end point. **Purified** material and crude ether extracts were **analyzed** by gas chromatography/**mass spectrometry** and nuclear magnetic resonance spectroscopy. The time of elution of MDF from both testicular and peritoneal macrophages was identical on all three HPLC columns. A single peak was observed when MDF, obtained from the final HPLC column, was **analyzed** by gas chromatography. The MS fragmentation pattern of **purified** material from both peritoneal and testicular macrophages was identical to that of a reference preparation of 25-hydroxycholesterol. Also, the nuclear magnetic resonance spectrum of MDF was similar to that of authentic 25-hydroxycholesterol. When 25-hydroxycholesterol was subjected to the identical **purification** scheme as MDF, it was found to elute at the same times as MDF on all three columns and elicited activity in the Leydig cell bioassay as expected. Control medium **purified** identically did not contain 25-hydroxycholesterol or have biological activity. Ether extracts of testis contained 25-hydroxycholesterol, indicating that this compound is present under physiological conditions. Similarly, when 25-hydroxycholesterol was injected into the testis of adult rats, **testosterone** production was increased within 3 h. Taken together, these data indicate that the lipophilic factor produced by macrophages that stimulates steroidogenesis is 25-hydroxycholesterol.

L31 ANSWER 16 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 2000:265490 BIOSIS
DOCUMENT NUMBER: PREV200000265490
TITLE: Characterization of exogenous **testosterone** in livestock by gas chromatography/combustion/isotope ratio **mass spectrometry**: Influence of feeding and age.
AUTHOR(S): Ferchaud, Veronique [Reprint author]; Le Bizec, Bruno; Monteau, Fabrice; Andre, Francois
CORPORATE SOURCE: LDH-LNR, Ecole Nationale Veterinaire (Ministere de l'Agriculture), F-44307, Nantes Cedex 3, France
SOURCE: Rapid Communications in Mass Spectrometry, (**2000**) Vol. 14, No. 8, pp. 652-656. print.
CODEN: RCMSEF. ISSN: 0951-4198.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Jun 2000
Last Updated on STN: 5 Jan 2002

AB The **detection** of exogenous **testosterone** in bovine urine was investigated by using gas chromatography/combustion/isotope

ratio **mass spectrometry** (GC/C/IRMS). The carbon isotopic ratio measurement of **epitestosterone**, etiocholanolone (**testosterone** metabolite) and DHEA (**testosterone** precursor) in female bovine urines after **testosterone** enanthate administration was carried out. An important modification in the 13C/12C ratio of **testosterone** metabolites was observed, such that significant differences between precursor and metabolites of **testosterone** occurred until three weeks after intramuscular administration of **testosterone** enanthate. The factors influencing the 13C/12C of endogenous steroids were studied especially through cattle feeding and age. The DHEA mean delta13C value was found to vary between -25 and -26%permill when hay and concentrate diet were used for fattening. On the other hand the delta13C value observed when maize silage was used increased to -20%permill. **Testosterone** metabolites showed the same delta13C increase as their precursor. Moreover, we observed a clear relationship between age and efficiency of misuse **determination.** Indeed, because of the lower concentration of natural hormones in young animals, the contribution of exogenous molecules increases significantly compared with older subjects. Consequently, demonstration of administration is easier to achieve in calves than in mature animals.

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ACCESSION NUMBER: 2000052651 EMBASE
TITLE: Quantitation of anabolic hormones and their metabolites in bovine serum and urine by **liquid chromatography**-tandem **mass spectrometry.**
AUTHOR: Draisci R.; Palleschi L.; Ferretti E.; Lucentini L.; Cammarata P.
CORPORATE SOURCE: R. Draisci, Laboratorio di Medicina Veterinaria, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Rome, Italy. draisci@iss.it
SOURCE: Journal of Chromatography A, (**2000**) 870/1-2 (511-522).
Refs: 26
ISSN: 0021-9673 CODEN: JCRAEY
PUBLISHER IDENT.: S 0021-9673(99)01293-5
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A specific and sensitive method based on tandem **mass spectrometry** with on-line high-performance **liquid chromatography** using atmospheric pressure chemical ionisation (LC-APCI-MS-MS) for the quantitation of anabolic hormone residues (17β-19- **nortestosterone**, 17β- **testosterone** and progesterone) and their major metabolites (17α-19- **nortestosterone** and 17α- **testosterone**) in bovine serum and urine is reported. [2H2]17β- **Testosterone** was used as internal standard. The **analytes** were extracted from urine (following enzymatic hydrolysis) and serum samples by liquid-liquid extraction and **purified** by C18 solid-phase extraction. Ionisation was performed in a heated nebulizer interface operating in the positive ion mode, where only the protonated molecule, [M+H]+, was generated for each **analyte.** This served as precursor ion for collision-induced dissociation and two diagnostic product ions for each **analyte** were identified for the unambiguous hormone confirmation

by selected reaction monitoring LC-MS-MS. The overall inter-day precision (relative standard deviation) ranged from 6.37 to 2.10% and from 6.25 to 2.01%, for the bovine serum and urine samples, respectively, while the inter-day accuracy (relative error) ranged from -5.90 to -3.18% and from -6.40 to -2.97%, for the bovine serum and urine samples, respectively. The limit of quantitation of the method was 0.1 ng/ml for all the hormones in bovine serum and urine. On account of its high sensitivity and specificity the method has been successfully used to confirm illegal hormone administration for regulatory purposes. Copyright (C) 2000.

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ACCESSION NUMBER: 2000013867 EMBASE
TITLE: Compared interest between hair **analysis** and urinalysis in doping controls: Results for amphetamines, corticosteroids and anabolic steroids in racing cyclists.
AUTHOR: Gaillard Y.; Vayssette F.; Pepin G.
CORPORATE SOURCE: Y. Gaillard, Laboratoire TOXLAB, 18 Rue Andre Del Sarte, 75018 Paris, France
SOURCE: Forensic Science International, (**2000**) 107/1-3 (361-379).
Refs: 28
ISSN: 0379-0738 CODEN: FSINDR
PUBLISHER IDENT.: S 0379-0738(99)00179-6
COUNTRY: Ireland
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 030 Pharmacology
035 Occupational Health and Industrial Medicine
037 Drug Literature Index
049 Forensic Science Abstracts
LANGUAGE: English
SUMMARY LANGUAGE: English

AB In France during a famous bicycle race, the newspapers documented the degree in which doping seemed to be supervised in some teams by managers and doctors. Use of anabolic steroids and other substances was officially banned in the mid-seventies by sports authorities. This policy has been enforced through urine testing before competition. It is well known, however, that a latency period is all that is necessary to defeat these tests. Nevertheless, hair **analysis** could be a promising tool when testing for periods that are not accessible to urinalysis any more. We have developed different sensitive methods for testing hair for amphetamines, anabolic steroids and their esters and corticosteroids. For amphetamines, 50 mg of hair were digested with 1 M NaOH, extracted with ethyl acetate, derivatized with TFA and **analyzed** by gas chromatography positive chemical-ionization **mass spectrometry.** For corticosteroids, 50 mg of powdered hair were treated with methanol in an ultrasonic bath and subsequently **purified** using a C18 solid phase extraction column. **Analysis** was realized by high performance **liquid chromatography** coupled to electrospray-ionization tandem **mass spectrometry.** For anabolic steroids and their esters, 100 mg of powdered hair were treated with methanol in an ultrasonic bath for extraction of esters, then alkaline digested with 1 M NaOH for an optimum recovery of other drugs. The two liquid preparations were subsequently extracted with ethyl acetate, pooled, then finally highly **purified** using a twin solid phase extraction on aminopropyl and silica cartridges. Residue was derivatized with MSTFA prior to injection. **Analysis** was conducted by gas chromatography coupled to a triple quadrupole **mass spectrometer.** Thirty cyclists were sampled and tested both in hair and in urine.

Amphetamine was **detected** 10 times in hair (out of 19 **analyses**) compared to 6 times in urine (out of 30 **analyses**). Corticosteroids were **detected** 5 times in hair (methylprednisolone 1 case, triamcinolone acetonide 3 cases and hydrocortisone acetate 1 case) in hair (out of 12 **analyses**) compared to 12 times (triamcinolone acetonide 10 cases and betamethasone 2 cases) in urine (out of 30 **analyses**). Anabolic steroids were **detected** twice (nandrolone 1 case, and **testosterone** undecanoate 1 case) in hair (out of 25 **analyses**) compared to none in urine (out of 30 **analyses**). Copyright (C) 2000 Elsevier Science Ireland Ltd.

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ACCESSION NUMBER: 2000:226073 BIOSIS
DOCUMENT NUMBER: PREV200000226073
TITLE: Concepts for the syntheses of biotinylated steroids. Part I: **Testosterone** derivatives as immunochemical probes.
AUTHOR(S): Hauptmann, Hagen; Paulus, Birgit; Kaiser, Thomas; Herdtweck, Eberhardt; Huber, Erasmus; Luppa, Peter B. [Reprint author]
CORPORATE SOURCE: Institute for Clinical Chemistry and Pathobiochemistry, Klinikum rechts der Isar, Technische Universitaet Muenchen, D-81675, Muenchen, Germany
SOURCE: Bioconjugate Chemistry, (**March-April, 2000**) Vol. 11, No. 2, pp. 239-252. print.
CODEN: BCCHES. ISSN: 1043-1802.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 7 Jun 2000
Last Updated on STN: 5 Jan 2002

AB We describe synthetic strategies for the biotinylation of **testosterone** (T) at positions 3, 7alpha, 17alpha, and 19. These T probes are able to mimic ligand binding and may provide for a better understanding of the biospecific interaction with steroid-binding proteins such as the androgen receptor, anti-steroid antibodies, or steroid-binding serum globulins. For the 7alpha- and 17alpha-derivatives, biotinyl-N-hydroxysuccinimide esters with different types of spacer chains were used. The 3-biotin hydrazone derivative was produced using N-(epsilon-biotinyl)-caproyl hydrazide, whereas for the 19-biotinylation, a biotinyl-1-N-diamino-3,6-dioxaoctane-amide was applied. Key reaction for the biotinylation at position 3 is the oximation of the 3-oxo function. The 17alpha-position is accessible by the reaction of the 3-protected 4-androsten-17-epoxide with oxygen in the beta-position, followed by nucleophilic ring opening with cyanide which provides the 17alpha-cyanomethyl derivative. The key step is the regioselective ketal protection of the 3-oxo function of androst-4-ene-3,17-dione using a stannoxane catalyst. An alternative pathway for the insertion of biotin at the 19-position was established by the synthesis of 17beta-hydroxy-androst-4-en-3-one-19-yl carboxymethyl ether. After activation by the carbodiimide method, the compound reacts with aminoterminal biotin derivatives. The copper(I)-catalyzed 1,6 Michael addition of 17-acetoxy-6,7-dehydro-T leads to 7alpha-derivatives by use of omega-silyl protected hydroxylalkyl-modified Grignard reagents. A functional group interconversion using the Staudinger reaction transforms the azide function into a primary omega-amino group. The absolute configurations of the different biotinylated derivatives were investigated by 1H NMR studies. For the 7alpha-biotinylated T series, additionally, an X-ray **analysis** proved the axial position of the spacer group.

This results in a vertical orientation of the biotin moiety toward the alpha-face of the planar tetracyclic backbone. Thus, a negligible alteration of the original structure of the upper beta-face offers the feasibility of applying the 7alpha-derivatives as optimal immunochemical tracers in competitive immunoassays. Biotinylated T derivatives should be also suitable for ligand-binding studies to the androgen receptor or to sex hormone-binding globulin.

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ACCESSION NUMBER: 2001:4515 BIOSIS
DOCUMENT NUMBER: PREV200100004515
TITLE: Biosynthesis of 6beta-**hydroxymethyltestosterone** using bovine hepatocyte cultures.
AUTHOR(S): Clouet-Dumas, Anne-Sophie [Reprint author]; Le Bizec, Bruno; Le Pape, Marie-Annick; Maume, Daniel; Monteau, Fabrice; Andre, Francois
CORPORATE SOURCE: LDH-LNR, Ecole Nationale Veterinaire (Ministere de l'Agriculture), F-44307, Nantes Cedex 3, France labolnr@vet-nantes.fr
SOURCE: Journal of Steroid Biochemistry and Molecular Biology, (**September, 2000**) Vol. 74, No. 1-2, pp. 57-62. print.
CODEN: JSBBEZ. ISSN: 0960-0760.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Dec 2000
Last Updated on STN: 21 Dec 2000

AB Usually performed to investigate biotransformations of xenobiotics, in vitro liver models could become useful tools for the synthesis of not commercially available compounds. In this study, bovine hepatocyte cultures were used to biosynthesise, on the laboratory scale, one major metabolite of **methyltestosterone**: 6beta-**hydroxymethyltestosterone.** After incubation of bovine hepatocytes with **methyltestosterone** for 24 h, culture medium was removed and stored at -20degreeC until **analysis.** The sample was extracted and **purified** on a reversed-phase HPLC system. The metabolite of interest was then **analysed** in LC-MS and GC-MS for structural identification. The purity and the isomery of the 6 and 17 positions were confirmed by NMR **analyses.** This first success in producing **purified** 6beta-**hydroxymethyltestosterone** from bovine hepatocyte cultures allowed us to consider that in vitro liver models could be reliable tools for standard biosynthesis.

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ACCESSION NUMBER: 1999311451 EMBASE
TITLE: Identification of **testosterone** and **testosterone** esters in human hair.
AUTHOR: Kintz P.; Cirimele V.; Jeanneau T.; Ludes B.
CORPORATE SOURCE: P. Kintz, Institut de Medecine Legale, 11 rue Humann, 67000 Strasbourg, France
SOURCE: Journal of Analytical Toxicology, (**1999**) 23/5 (352-356).
Refs: 18
ISSN: 0146-4760 CODEN: JATOD3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
029 Clinical Biochemistry

037 Drug Literature Index
040 Drug Dependence, Alcohol Abuse and Alcoholism
LANGUAGE: English
SUMMARY LANGUAGE: English
AB In 1974, steroids were added to the list of doping agents banned by the International Olympic Committee because of their effects on the performance of the athletes. **Testosterone** and its esters promote the development of secondary male sexual characteristics and accelerate muscle growth. The mandatory test to **detect testosterone** abuse is to measure the ratio of **testosterone** to **epitestosterone** in the urine. However, because athletes can adjust their dosage to stay within the range permitted, there is a risk of test evasion. Therefore, we developed two original procedures to **determine testosterone** and its esters in human hair. First, **testosterone** was investigated in hair obtained from 26 control subjects. After decontamination with dichloromethane, 100 mg of hair was incubated in 1M NaOH in the presence of 1 ng of **testosterone**-d3. After neutralization, the extract was **purified** using solid-phase extraction with isolute C18 columns followed by liquid- liquid extraction with pentane. After silylation, **testosterone** was **analyzed** by gas chromatography-**mass spectrometry.** Concentrations were in the range 1.2 to 11.4 pg/mg with a mean value of 3.8 pg/mg. To distinguish exogenous abuse from endogenous levels, the incorporation of **testosterone** esters into hair was investigated. Preparation involved methanolic incubation to avoid the cleavage of the esters. In a panel of eight esters, it was possible to identify **testosterone** propionate, **testosterone** enanthate, and **testosterone** decanoate in the hair of two bodybuilders and one weight lifter. This new technology may find useful applications in anabolic abuse control.

L31 ANSWER 22 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 1999:256450 BIOSIS
DOCUMENT NUMBER: PREV199900256450
TITLE: Supercritical fluid extraction of **methyltestosterone**, **nortestosterone** and **testosterone** at low ppb levels from fortified bovine urine.
AUTHOR(S): Stolker, A. A. M.; van Ginkel, L. A.; Stephany, R. W.; Maxwell, R. J. [Reprint author]; Parks, O. W.; Lightfield, A. R.
CORPORATE SOURCE: Eastern Regional Research Center, US Department of Agriculture, ARS, 600 E. Mermaid Lane, Wyndmoor, PA, 19038, USA
SOURCE: Journal of Chromatography B, (**April 16, 1999**) Vol. 726, No. 1-2, pp. 121-131. print. CODEN: JCBADL. ISSN: 0378-4347.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Jul 1999
Last Updated on STN: 2 Jul 1999
AB A multi-residue supercritical fluid extraction (SFE) method is proposed for the isolation of **nortestosterone**, **testosterone** and **methyltestosterone** from bovine urine. Prior to SFE, bovine urine was hydrolyzed and then fortified with the three steroids at 100 ng/ml and 50 ng/ml each for HPLC **analysis** and 25 ng/ml and 12.5 ng/ml each for GC-MS **analysis.** The samples then were mixed with an adsorbent material, placed in an SFE extraction vessel prepacked with a 3-ml SPE column containing neutral alumina and the **testosterones**

were extracted from the urine matrix using unmodified supercritical CO2 at 27.2 MPa and 40degreeC. The steroids were retained in-line on the neutral alumina sorbent in the SPE column while co-extracted artifactial material was trapped off-line after CO2 decompression. After SFE, the SPE column was removed from the extraction vessel, and the trapped steroids were eluted from the neutral alumina sorbent with 3 ml of a methanol-water mixture. Eluates were used directly without post-SFE clean-up either for HPLC **analysis** (**detection** limit 50 ng/ml) or for GC-MS **analysis** (**detection** limit 5 ng/ml after steroid derivatization). The multi-residue SFE recoveries (n=6) for **nortestosterone**, **testosterone** and **methyltestosterone** from hydrolyzed bovine urine by GC-MS **analysis** were 90.8+-6%, 93.9+-3% and 92.5+-5%, respectively for each steroid at the 12.5 ng fortification level.

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ACCESSION NUMBER: 1998:276203 BIOSIS
DOCUMENT NUMBER: PREV199800276203
TITLE: 4-**Chlorotestosterone** acetate metabolites in cattle after intramuscular and oral administrations.
AUTHOR(S): Le Bizec, Bruno; Montrade, Marie-Pierre; Monteau, Fabrice; Gaudin, Isabelle; Andre, Francois [Reprint author]
CORPORATE SOURCE: LDH-LNR, Ecole Nationale Veterinaire, BP 50707, 44307 Nantes Cedex 03, France
SOURCE: Clinical Chemistry, (**May, 1998**) Vol. 44, No. 5, pp. 973-984. print.
CODEN: CLCHAU. ISSN: 0009-9147.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Jun 1998
Last Updated on STN: 13 Aug 1998

AB The use of 4-**chlorotestosterone** acetate by farmers for cattle fattening was recently demonstrated although the use of this anabolic steroid is strictly forbidden in the European Union. We investigated the metabolism of 4-**chlorotestosterone** acetate in the bovine species after intramuscular and oral administration. Nineteen metabolites were **detected** in urine after intramuscular injection, and eight metabolites were identified. For this purpose, preparative HPLC, **mass spectrometry** with different ionization modes (electronic impact and chemical ionization), and different acquisition techniques were used (high resolution, selected ion monitoring, and scan measurement). Metabolite stereoisomerism was **determined** on the basis of retention time and organic synthesis. 4-**Chloroepitestosterone** (M2), 4-chloroandrost-4-en-3alpha-ol-17-one (M3), and 4-chloroandrost-4-ene-3,17-dione (M4) were identified as the main urinary markers of intramuscular administration. On the other hand, 4-chloroandrost-4-ene-3alpha,17beta-diol (M7), 4-chloroandrostan-3beta-ol-17-one (M5), and M2 were the primary indicators of an oral administration. In addition, we have shown that 95% of the metabolites were sulfo-conjugated, except for M3, which was partially conjugated to glucuronic acid. Finally, the main metabolites (M2, M3, and M4) were easily identified for 1.5 months after intramuscular administration.

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ACCESSION NUMBER: 1998034359 EMBASE
TITLE: Bovine adrenals contain, in addition to ouabain, a second inhibitor of the sodium pump.
AUTHOR: Schneider R.; Wray V.; Nimtz M.; Lehmann W.D.; Kirch U.;

Antolovic R.; Schoner W.
CORPORATE SOURCE: W. Schoner, Inst. fur Biochemie/Endokrinologie, Fachbereich Veterinarmedizin, Justus-Liebig-Universitat Giessen, Frankfurter Strasse 100, D-35392 Giessen, Germany. Schoner@vetmed.uni-giessen.de
SOURCE: Journal of Biological Chemistry, (**1998**) 273/2 (784-792).
Refs: 30
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 003 Endocrinology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB In the search for endogenous cardiac glycosides, two different inhibitors of the sodium pump have been isolated from bovine adrenals. Inhibitor A with a molecular mass of 600 Da and a UV maximum at 250 nm was **purified** from 16 kg of bovine adrenals. The pure substance (<1 ng) inhibited the sodium pump of human red blood cells with an affinity similar to that of ouabain, yet it cross-reacted with antibodies against the bufadienolide proscillaridin A but not against the cardenolide ouabain. Inhibitor A was slightly more hydrophilic than ouabain on RP-C18 high pressure **liquid chromatography.** Hence, it showed properties similar to the proscillaridin A immunoreactivity (Sich, B., Kirch, U., Tepel, M., Zideck, W., and Schoner, W. (1996) Hypertension 27, 1073-1078) that increased in humans with systolic blood pressure and pulse pressure. Inhibitor B of the sodium pump with a molecular mass of 584 Da was **purified** 106-fold from 20 kg of bovine adrenals. It cross-reacted with antibodies against ouabain but not with antibodies against proscillaridin A and inhibited the sodium pump of human and rat red blood cells with the same affinity as ouabain. All other properties, such as the retention time in a C18-reversed phase chromatography, molecular mass **determination** by electrospray **mass spectrometry** and fragmentation pattern, and UV and 1H NMR spectroscopic data, were identical to ouabain. Hence, sodium pump inhibitor B from bovine adrenals is the cardenolide ouabain.

L31 ANSWER 25 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 1997:405337 BIOSIS
DOCUMENT NUMBER: PREV199799711540
TITLE: Confirmation of anabolic hormone residues in bovine blood by micro-HPLC-ion spray-tandem **mass spectrometry.**
AUTHOR(S): Draisci, Rosa [Reprint author]; Giannetti, Luigi; Lucentini, Luca; Purificato, Luca Vv Palleschiana; Moretti, Gabriella
CORPORATE SOURCE: Lab. di Med. Veterinaria, Ist. Superiore di Sanita, v.le Regina Elena 299, 00161 Roma, Italy
SOURCE: HRC Journal of High Resolution Chromatography, (**1997**) Vol. 20, No. 8, pp. 421-430.
CODEN: JHRCE7. ISSN: 0935-6304.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Sep 1997
Last Updated on STN: 21 Nov 1997

AB Sensitive, specific **analytical** methods for the **determination** of anabolics in biological matrices are essential to control the illegal use of these substances in food-producing animals.

Programs of residue control are performed annually in Italy for the **determination** of endogenous sex hormones (17-beta-estradiol, progesterone, **testosterone**) for which maximum physiological levels have been established. At present, the methods used in the Italian programs to **determine** natural hormones in bovine blood are based on the sensitive radioimmunoassay (RIA), due to relatively low levels of these substances in plasma/serum. In this study, we report a new method based on tandem **mass spectrometry** with on-line micro-high performance **liquid chromatography** (micro-HPLC-MS-MS) using an atmospheric pressure ionization (API) source and an ion spray (IS) interface for the specific direct **detection** of natural (progesterone and **testosterone**), and synthetic (17-beta-19-**nortestosterone** and medroxyprogesterone) hormone residues in bovine serum. 17-**Methyltestosterone** was used as the internal standard. **Analytes** were extracted with acetate buffer, **purified** on C18 Solid Phase Extraction (SPE) cartridge and separated on a reverse phase C18 microHPLC column (300 mm times 1 mm, 5 gm), using acetonitrile-water, 80:20 (v/v) containing 2 mM ammonium acetate as the mobile phase, at a flow rate of 10 mu-l/min. When anabolic hormones were ionized in the IS interface operating in the positive ion mode, only the protonated molecules, (M+H)+, were generated, without evidence of any fragmentation. These served as precursor ions for collision induced dissociation (CID) and diagnostic daughter ions for each **analyte** were identified in order to carry out **analysis** by microHPLC-MS-MS in the selected reaction monitoring (SRM) mode. For the **analytes** in question, the response of the mass **detector** was related linearly to the quantity of each **analyte** injected between 10 and 300 pg, in the SRM mode. The limit of **detection**, based on a 3:1 signal-to-noise ratio, is 6-7 pg for the hormones. Recoveries were higher than 83% for 17-beta-19-**nortestosterone**, **testosterone**, and 17-**methyltestosterone**, and 72% for medroxyprogesterone, and progesterone. The micro-HPLC-MS-MS method for the **determination** of anabolic hormones in bovine blood requires no sample derivatization, minimal sample preparation, and provides a sensitive, selective, rapid alternative to the existing **purification**, separation, and **detection** techniques. At present this very sensitive method is being successfully applied to measure bovine serum concentrations of natural hormones, such as **testosterone** and progesterone, in order to then confirm any illegal administration of these substances.

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ACCESSION NUMBER: 97307249 EMBASE
DOCUMENT NUMBER: 1997307249
TITLE: Specific CYP3A4 inhibitors in grapefruit juice: Furocoumarin dimers as components of drug interaction.
AUTHOR: Fukuda K.; Ohta T.; Oshima Y.; Ohashi N.; Yoshikawa M.; Yamazoe Y.
CORPORATE SOURCE: K. Fukuda, Pharmaceutical Developm. Res. Lab., Tanabe Seiyaku Co. Ltd, 2-2-50 Kawagishi, Toda-shi, Saitama 335, Japan. k-fukuda@tanabe.co.jp
SOURCE: Pharmacogenetics, (**1997**) 7/5 (391-396).
Refs: 30
ISSN: 0960-314X CODEN: PHMCEE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
037 Drug Literature Index
LANGUAGE: English

SUMMARY LANGUAGE: English

AB Four components were isolated from grapefruit juice that: inhibit human CYP3A-mediated drug oxidation. The structures of these compounds were identified as furocoumarin derivatives by absorption spectra, APCI-**liquid chromatography**/tandem **mass spectrometry** and nuclear magnetic resonance after their **purification** by reversed-phase high performance **liquid chromatography.** They include two new furocoumarins, 4-[[6-hydroxy-7-[[1-[(1-hydroxy-1-methyl) ethyl]-4-methyl-6-(7-oxo-7H-furo[3,2-g][1]benzopyran-4-yl)-4-hexenyl]oxy]-3,7 -dimethyl-2-octenyl] oxy]-7H-furo[3,2-g][1]benzopyran-7-one (GF-I-1) and 4-[[6-hydroxy-7-[[4-methyl-1-(1-methylethenyl)-6-(7-oxo-7H-furo[3,2 -g][1]benzopyran-4-yl)-4-hexenyl]oxy]-3,7-dimethyl-2-octenyl]oxy]-7H-furo[3,2 -g][1]benzopyran-7-one (GF-I-4). These furocoumarins are strong candidates for causative agents of grapefruit juice-mediated drug interaction, because of an inhibition potential that is equal to or stronger than the prototypical CYP3A4 inhibitor, ketoconazole, on liver microsomal **testosterone** 6β-hydroxylation.

L31 ANSWER 27 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 5

ACCESSION NUMBER: 1997:199000 BIOSIS
DOCUMENT NUMBER: PREV199799498203
TITLE: Confirming **testosterone** administration by isotope ratio **mass spectrometric analysis** of urinary androstanediols.
AUTHOR(S): Shackleton, Cedric H. L. [Reprint author]; Phillips, Andy; Chang, Tony; Li, Ye
CORPORATE SOURCE: Children's Hosp. Oakland Res. Inst., 747 52nd St., Oakland, CA 94609, USA
SOURCE: Steroids, (**1997**) Vol. 62, No. 4, pp. 379-387. CODEN: STEDAM. ISSN: 0039-128X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 12 May 1997
Last Updated on STN: 2 Jun 1997

AB A gas chromatographic combustion isotope ratio **mass spectrometric** (GC/C/IRMS) method was used for studying the incorporation of exogenous **testosterone** enanthate into excreted urinary 5-alpha- and 5-beta-androstane-3-alpha,17-beta-diols. A multistep but straightforward work-up procedure produced a simple GC chromatogram of urinary steroid acetates composed principally of two androstanediols and pregnanediol. It is anticipated that such a method may form the basis of a doping control test for **testosterone** that could be used as a primary method during major sporting events or alternatively as a verification technique. Urine samples from five individuals were collected before and after administration of **testosterone** enanthate (250 mg). The delta-13C degree /00 value of androstanediols was around -26 to -28 during the baseline period and decreased to about -29 to -30 in the days following synthetic **testosterone** administration. One of the other major steroids in the chromatogram, pregnanediol, was utilized as the "internal standard," because its delta-13C degree /00 values did not markedly change following **testosterone** administration, remaining at -25 to -27. In all subjects studied, the delta-13C degree /00 values for androstanediols were reduced sufficiently over 8 days to confirm administration of synthetic **testosterone.** Although steroids isolated from urine of normal individuals from 12 different countries gave values between -24 and -28, this seemed not to be related to nationality or region. The most likely variable is the proportion of plants with low and high carbon 13 content in the diet.

This variable is likely to be more affected by individual food preferences than broad ethnic food divisions. In this paper, we propose a ratio of delta-13C degree /00 for androstendiols to pregnanediol as a useful discriminant of **testosterone** misuse, a value above 1.1:1.0 being indicative of such misuse. The work-up procedure was designed for batch **analysis** and to use only simple techniques, rather than employ further instrumentation, such as high-performance **liquid chromatography** (HPLC), in **purifying** steroids for GC/C/IRMS.

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ACCESSION NUMBER: 1997:212056 BIOSIS
DOCUMENT NUMBER: PREV199799511259
TITLE: Direct **determination** of anabolic steroid conjugates in human urine by combined high-performance **liquid chromatography** and tandem **mass spectrometry.**
AUTHOR(S): Bean, Karen A.; Henion, Jack D. [Reprint author]
CORPORATE SOURCE: Analytical Toxicol., Diagn. Lab., Cornell Univ., 927 Warren Dr., Ithaca, NY 14850, USA
SOURCE: Journal of Chromatography B, (**1997**) Vol. 690, No. 1-2, pp. 65-75.
CODEN: JCBADL. ISSN: 0378-4347.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 12 May 1997
Last Updated on STN: 2 Jun 1997

AB A novel screening procedure for the sulfate and glucuronide conjugates of **testosterone** (T) and **epitestosterone** (E) in human urine was developed based on liquid-solid extraction and microbore high-performance **liquid chromatography** combined on-line with ion-spray tandem **mass spectrometry.** Confirmation of the sulfate and glucuronide conjugates of **testosterone** and **epitestosterone** isolated from normal human urine was achieved by selected reaction monitoring of characteristic product ions of the parent compounds. Endogenous levels of the steroid conjugates are **detected** in normal male urine and an increase is observed when the sample is fortified with authentic **analytical** standards of the conjugates. Calibration curves of all steroid conjugates in urine are linear over a range of twenty. Deuterated internal standards of **testosterone** glucuronide and **epitestosterone** sulfate were used for quantitation of the endogenous conjugates. T/E ratios were **determined** based on the glucuronide fractions of six replicates from a normal male and were shown to be statistically reproducible and below the accepted T/E threshold of 6:1. Sulfate conjugates were shown to be present at significantly lower levels in the urine. The method has potential as an alternative for monitoring anabolic steroid conjugates in human urine.

L31 ANSWER 29 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 6

ACCESSION NUMBER: 1997:60587 BIOSIS
DOCUMENT NUMBER: PREV199799359790
TITLE: **Detection** of **testosterone** misuse: Comparison of two chromatographic sample preparation methods for gas chromatographic-combustion/isotope ratio **mass spectrometric analysis.**
AUTHOR(S): Aguilera, R.; Becchi, M. [Reprint author]; Grenot, C.; Casabianca, H.; Hatton, C. K.

CORPORATE SOURCE: Serv. Central Anal., CNRS, BP 22, 69390 Vernaison, France
SOURCE: Journal of Chromatography B Biomedical Applications, (**1996**) Vol. 687, No. 1, pp. 43-53.
CODEN: JCBADL. ISSN: 0378-4347.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 11 Feb 1997
Last Updated on STN: 11 Feb 1997

AB Two chromatographic methods, reversed-phase **liquid chromatography** (LC) and immunoaffinity chromatography (IAC), were compared in the preparation of **purified testosterone** extracts suitable for gas chromatography-combustion/isotope ratio **mass spectrometry** (GC-C-IRMS) **analysis.** We have shown previously that GC-C-IRMS is a promising means of **detection** of **testosterone** misuse in sport. The two clean-up procedures afford sufficient recovery and adequate purity of **testosterone.** LC presents several advantages over IAC: access to other urinary steroids, longer column life, no need for special equipment and no antibody preparation. For IAC, the antibodies to **testosterone** must be selected with care for high affinity and low cross-reactivity. Nevertheless, IAC is of some interest in our experiments, the recovery is slightly better for low concentrations of urinary **testosterone** and IAC does not induce isotopic discrimination even in overloading experiments. This is the first report on sample preparation by IAC prior to GC-C-IRMS and carbon isotope ratio values for urinary **epitestosterone.** The carbon isotope ratio test can identify users' urines missed by the **testosterone** to **epitestosterone** ratio (T/E gt 6) test.

L31 ANSWER 30 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 7

ACCESSION NUMBER: 1995:446184 BIOSIS
DOCUMENT NUMBER: PREV199598460484
TITLE: A novel nonhepatic hydroxycholesterol 7-alpha-hydroxylase that is markedly stimulated by interleukin-1-beta: Characterization in the immature rat ovary.
AUTHOR(S): Payne, Donna W. [Reprint author]; Shackleton, Cedric; Tomas, Harold; Ben-Shlomo, Izhar; Kol, Shahar; Demoura, Marcos; Strauss, Jerome F.; Adashi, Eli Y.
CORPORATE SOURCE: Div. Reproductive Endocrinol., Univ. Maryland Sch. Med., 655 W. Baltimore St., Rm. 11-010, Baltimore, MD 21201, USA
SOURCE: Journal of Biological Chemistry, (**1995**) Vol. 270, No. 32, pp. 18888-18896.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Oct 1995
Last Updated on STN: 10 Oct 1995

AB During studies on the regulation of rat ovarian steroidogenic enzymes by interleukin-1-beta (IL-1-beta), we observed substantial metabolism of 25-hydroxycholesterol to two unusual polar products. This unexpected effect was observed both in isolated granulosa cells and in whole ovarian dispersates and was also induced by tumor necrosis factor alpha, but not by insulin-like growth factor I or follicle-stimulating hormone. The effect was dependent on time and the dose of IL-1-beta and was blocked by an IL-1 receptor antagonist. The formation of the polar metabolites was inhibited by ketoconazole and trilostane, but not by aminoglutethimide. Subsequent **purification** of these novel metabolites and **analysis** by gas chromatography/**mass spectrometry** , NMR, and high performance **liquid chromatography**

revealed them to be related 7-alpha-hydroxylated hydroxycholesterols (cholest-4-ene-7-alpha,25-diol-3-one and cholest-5-ene-3-beta,7-alpha,25-triol). IL-1-beta-stimulated ovarian 7-alpha-hydroxylase activity (3-10 pmol/min/mg of cellular protein) was nearly 4-fold that of control levels using 25-hydroxycholesterol as substrate. Activities at or below control levels were observed when IL-1-beta-treated cell sonicates were boiled or assayed in the presence of NADH (rather than NADPH), indicating that involvement of a nonenzymatic process was unlikely. IL-1-beta-stimulated 7-alpha-hydroxylase activity was inhibited to basal levels by a 10-fold excess of unlabeled 25- or 27-hydroxycholesterol, but not by cholesterol, pregnenolone, progesterone, **testosterone**, or dehydroepiandrosterone, suggesting that ovarian 7-alpha-hydroxylase is specific for hydroxycholesterols. Furthermore, when IL-1-beta-treated ovarian cultures were incubated with radiolabeled cholesterol or **testosterone**, no 7-alpha-hydroxylated products were observed. We were also unable to **detect** any mRNA transcripts for liver cholesterol 7-alpha-hydroxylase in IL-1-beta-stimulated ovarian cultures. This study describes an ovarian hydroxycholesterol 7-alpha-hydroxylase that differs from liver cholesterol 7-alpha-hydroxylase and from other nonhepatic progestin/androgen 7-alpha-hydroxylases. The novel finding of the regulation of a 7-alpha-hydroxylase by IL-1-beta (and tumor necrosis factor alpha) suggests a unique role for cytokines in the regulation of cholesterol metabolism in the ovary and possibly other tissues.

L31 ANSWER 31 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 1995:482893 BIOSIS
DOCUMENT NUMBER: PREV199598497193
TITLE: Methods for urinary **testosterone analysis**
.
AUTHOR(S): Venturelli, Elisabetta [Reprint author]; Cavalleri, Adalberto; Secreto, Giorgio
CORPORATE SOURCE: National Cancer Inst., Endocrine Unit, via Venezian 1, 20133 Milano, Italy
SOURCE: Journal of Chromatography B Biomedical Applications, (**1995**) Vol. 671, No. 1-2, pp. 363-380.
CODEN: JCBADL. ISSN: 0378-4347.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 9 Nov 1995
Last Updated on STN: 9 Nov 1995

AB Urinary **testosterone analysis** requires a multistep procedure to achieve a good degree of sensitivity and specificity in the dosage. Hydrolysis, extraction, **purification** and quantification are usually performed in sequence, and several options can be chosen for each of them. After introductory remarks on the applications of urinary **testosterone** measurement and a short description of the metabolic pathway of the hormone, an overview of the techniques most commonly used in each step is presented. Advantages and disadvantages of each of them are outlined, and a procedure for urinary **testosterone analysis** is suggested. The procedure consists of: enzymatic hydrolysis with Helix pomatia juice, followed by solid-phase extraction of hydrolyzed urine by a C-18 cartridge coupled with an NH-2 cartridge and high-performance **liquid chromatography** cleanup of the extract. Then, quantification can be achieved by gas chromatography or radioimmunoassay.

L31 ANSWER 32 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 1995:547459 BIOSIS
DOCUMENT NUMBER: PREV199698561759
TITLE: Isocratic high-performance **liquid chromatographic** method for the separation of **testosterone** metabolites.
AUTHOR(S): Sanwald, Patricia; Blankson, Evelyn A.; Dulery, Bertrand D.; Schoun, Josiane; Huebert, Norman D.; Dow, James [Reprint author]
CORPORATE SOURCE: Dep. Drug Metabolism, Marion Merrell Dow, 16 Rue d'Ankara, 67080 Strasbourg, Cedex, France
SOURCE: Journal of Chromatography B Biomedical Applications, (**1995**) Vol. 672, No. 2, pp. 207-215.
CODEN: JCBADL. ISSN: 0378-4347.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 31 Dec 1995
Last Updated on STN: 31 Dec 1995

AB An isocratic reversed-phase high-performance **liquid chromatographic** (HPLC) method using an Ultrasphere IP column has been developed for the **determination** of **testosterone** and its metabolites after incubation of 4-14C-labelled or unlabelled **testosterone** with rat liver microsomes. Compounds were eluted with methanol-watertetrahydrofuran (35:55:10, v/v, pH 4.0) and **detected** by ultraviolet (UV) absorption at 245 nm. UV or on-line radioactivity **detection** can be used although, due to differences in **detector** cell volumes, peak resolution is slightly better with UV **detection.** Selectivity was validated by collecting HPLC peaks and verifying their identity by gas chromatography-**mass spectrometry** after derivatization by N,O-bis(trimethylsilyl)trifluoroacetamide-trimethylchlorosilane. A three-day validation was performed to **determine** the linearity, repeatability, reproducibility and accuracy of the method, using corticosterone as internal standard. The method is applicable to the measurement of cytochrome P-450 isoenzyme activities in rat liver.

L31 ANSWER 33 OF 49 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 94044634 EMBASE
DOCUMENT NUMBER: 1994044634
TITLE: In vitro biotransformation of finasteride in rat hepatic microsomes: Isolation and characterization of metabolites.
AUTHOR: Ishii Y.; Mukoyama H.; Ohtawa M.
CORPORATE SOURCE: Banyu Pharmaceutical Co., Ltd., 810, Nishijo, Menuma-machi,Osato-gun, Saitama 360-02, Japan
SOURCE: Drug Metabolism and Disposition, (**1994**) 22/1 (79-84).
ISSN: 0090-9556 CODEN: DMDSAI
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Metabolism of finasteride ([N-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androst- 1-ene-17β-carboxamide]; MK-906), a new type of specific inhibitor of **testosterone** 5α-reductase, was investigated using rat hepatic microsomes. The metabolism of finasteride by rat hepatic microsomes was oxygen- and NADPH-dependent, and addition of metyrapone, 7,8-benzoflavone, and cytochrome c to the incubation mixture inhibited the metabolism of finasteride. It is suggested that the metabolic reaction of

finasteride was mediated by a mixed function oxidase involving P-450. Four major metabolites were **detected** in vitro on incubating finasteride with hepatic microsomes of rats treated with phenobarbital (PB-Ms), whereas two major metabolites were found in the incubation mixture with microsomes of untreated rats (UT-Ms). These metabolites were isolated and **purified** by solvent extraction and semi-preparative HPLC, and identified by MS spectrometry and NMR spectroscopy. The metabolites consisted of ω-hydroxy finasteride (M-1), finasteride-ω-al (M- 2), finasteride-ω-oic acid (M-3), and 6α-OH finasteride (M-4). M-1 and M-4 are the major metabolites in UT-Ms, and M-1 and M-3 in PB-Ms. These studies revealed that hydroxylation of the t-butyl group and ring hydroxylation at the 6-position were key steps in the metabolism of finasteride in the rat hepatic microsomes. Further, the major metabolite M-4 was hydroxylated at the 6α-position, but not at the 6β-position of the drug. This finding suggests the existence of a novel enzyme that catalyzes the 6α-hydroxylation of the 4-azasteroid.

L31 ANSWER 34 OF 49 JICST-EPlus COPYRIGHT 2005 JST on STN
ACCESSION NUMBER: 950081719 JICST-EPlus
TITLE: **Analysis** of protein anabolic hormone in beef by APCI LC-MS.
AUTHOR: ITO MITSUO; AOKI NOBUZANE
CORPORATE SOURCE: Environ. and Health Res. Lab., City of Kobe
SOURCE: Kobeshi Kankyo Hoken Kenkyu Shoho (Annual Report of Public Health Research Institute of Kobe City), (1994) vol. 22, pp. 39-48. Journal Code: Y0084A (Fig. 9, Tbl. 4, Ref. 6) ISSN: 0910-7738
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
LANGUAGE: Japanese
STATUS: New
AB The use of APCI LC-MS in residue **analysis** of 16 anabolic agents is described. The compounds under investigation were (androgens): 19-Nortestosterone, **Testosterone**, 17A-Methyltestosterone, **Testosterone** propionate, (Gestagens): Medroxyprogesterone, Progesterone, Medroxyprogesterone acetate and (Estrogens): Zeranol, 17B-Estradiol, 17A-Estradiol, 17A-Ethynyl estradiol, Estrone, Diethylstilbestrol, Dienestrol, Hexstrol, 17B-Estradiol-3-benzoate. Anabolic agents were extracted and **purified** with Miyazaki's method, and they were separated into two fractions. First fraction contained ketosteroids and esters and the second contained estrogens. For the first fraction, liquid chlomatography-mas spectrometry was performed in the positive ionization mode. The **mass spectra** obtained were relatively simple, protonated molecular ions ¢M+H!+. Estrogens in the second fraction were **analyzed** with negative ionization mode. They also gave simple ions ¢M-H!-. The calibration curves with SIM were rectilinear from 1.0 to 50ng with the **detection** limits of 10-20ng/g for beef samples. (author abst.)

L31 ANSWER 35 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 8
ACCESSION NUMBER: 1993:386842 BIOSIS
DOCUMENT NUMBER: PREV199396062142
TITLE: Secretion of 19-hydroxyandrostenedione and 19-**hydroxytestosterone** by porcine Leydig cells in vitro and in vivo.
AUTHOR(S): Raeside, J. I. [Reprint author]; Renaud, R. L. [Reprint author]; Friendship, R. M.; Khalil, M. W.
CORPORATE SOURCE: Dep. Biomed. Sci., Univ. Guelph, Guelph, ON N1G 2W1, Canada

SOURCE: Journal of Endocrinology, (**1993**) Vol. 137, No. 2, pp. 281-289.
CODEN: JOENAK. ISSN: 0022-0795.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Aug 1993
Last Updated on STN: 28 Sep 1993

AB 19-**Hydroxytestosterone** and 19-hydroxyandrostenedione have been identified as secretory products of the testes in the mature male domestic pig. Their isolation and identification were made by reverse-phase high-performance **liquid chromatography** and capillary gas chromatography-**mass spectrometry** (CGC-MS) of extracts from testicular vein blood and media of incubations with Leydig cells. Blood was collected from veins on the surface of the testes of anaesthetized boars. Collagenase-dispersed Percoll-**purified** cells (gt 90% pure) were incubated (20 times 106 cells/flask) with androstenedione (8.75 mu-mol/l) or (3H)androstenedione (5 times 10-6 c.p.m.) for lt 60 min. Steroids were recovered from plasma or media by solid-phase extraction and the unconjugated fractions chromatographed isocratically in two solvent systems (acetonitrile:water, 37:63 (v/v) and methanol:water, 70:30 (v/v)) before CGC-MS **analysis.** 19-**Hydroxytestosterone** was present in greater quantities than 19-hydroxyandrostenedione in testicular vein blood; it was also seen as a quantitatively significant metabolite of unlabelled and radioactive androstenedione in the incubation studies. The demonstration of the secretion of 19-hydroxyandrogens from porcine testes thus raises questions concerning the physiological significance of a testicular, rather than an adrenal, secretion of these compounds.

L31 ANSWER 36 OF 49 EMBASE on STN

ACCESSION NUMBER: 93089853 EMBASE
DOCUMENT NUMBER: 1993089853
TITLE: Metabolism of 17β,19- **nortestosterone** in urine of calves after oral intake and intramuscular administration.
AUTHOR: Daeseleire E.; De Guesquiere A.; Van Peteghem C.
CORPORATE SOURCE: Laboratory of Food Analysis, University of Ghent, Harelbekestraat 72,9000 Ghent, Belgium
SOURCE: Analytica Chimica Acta, (**1993**) 275/1-2 (95-103).
ISSN: 0003-2670 CODEN: ACACAM
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The metabolism of 17β,19- **nortestosterone** was investigated in six calves. Three calves were injected with Laurabolin and the other three were fed 17β,19- **nortestosterone**-containing food. Urine samples were taken before and at regular time intervals after the injection or oral intake. After enzymatic hydrolysis, the sample clean-up consisted in solid-phase extraction followed by a **liquid chromatographic purification. Detection** was by gas chromatography-**mass spectrometry.** Urine samples from pregnant and non-pregnant cows and from steers were also collected and **analysed** in the same way.

L31 ANSWER 37 OF 49 BIOSIS on

STN DUPLICATE 9

ACCESSION NUMBER: 1992:500360 BIOSIS
DOCUMENT NUMBER: PREV199294118885; BA94:118885
TITLE: CHARACTERIZATION OF MET-139 AS THE PHOTOLABELED AMINO ACID RESIDUE IN THE STEROID BINDING SITE OF SEX HORMONE BINDING GLOBULIN USING DELTA-6 DERIVATIVES OF EITHER **TESTOSTERONE** OR ESTRADIOL AS UNSUBSTITUTED PHOTOAFFINITY LABELING REAGENTS.
AUTHOR(S): GRENOT C [Reprint author]; DE MONTARD A; BLACHERE T; ROLLAND DE RAVEL M; MAPPUS E; CUILLERON C Y
CORPORATE SOURCE: INST NATL DE LA SANTE ET DE LA RECHERCHE MEDICALE, UNITE INSERM U 329, PATHOLOGIE HORMONALE MOLECULARIE, HOPITAL DEBROUSSE, 69322 LYON, FRANCE
SOURCE: Biochemistry, (**1992**) Vol. 31, No. 33, pp. 7609-7621.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 9 Nov 1992
Last Updated on STN: 24 Dec 1992

AB **Immunopurified** human sex hormone binding globulin (SHBG) was photoinactivated and photolabeled by radioinert and radioactive photoaffinity labeling steroids Δ6- **testosterone** (Δ6-T) and Δ6-estradiol (Δ6-E2). The maximal levels of specific incorporation of these two reagents were 0.50 and 0.33 mol of label/mol of SHBG, respectively. Covalently labeled SHBG fractions were citraconylated, reduced, carboxymethylated, and cleaved by trypsin. Separation of tryptic digests by reverse-phase **liquid chromatography** gave single radioactive peaks at the same retention times with both steroid reagents. However, the two labeled peptidic fractions could be distinguished by capillary electrophoresis and **immunodetection** with anti-steroid antibodies, whereas the covalent attachment of radioactivity was confirmed by thin-layer chromatography on silica gel. Edman degradation of the two labeled peptides showed a single sequence His-Pro-Ile-([3H]X)-Arg corresponding to the pentapeptides His-Pro-Ile-Met-Arg 136-140 of SHBG sequence. The coincidence, in both cases, of the absence of an identifiable amino acid residue and of the elution of the most intense peak of radioactivity at the fourth cycle of Edman degradation suggests that the same Met-139 residue was labeled by Δ6-[1,2-3H2]T or by Δ6-[17α-3H]E2. Liquid secondary ion **mass spectrometry** of the two peptides showed [M + H]+ ions at m/z 939.8 or 923.8, corresponding respectively to the addition of Δ6-T or Δ6-E2 to the pentapeptide. The presence of the steroid molecule in the Δ6-[3H]T-pentapeptide conjugate was confirmed by the difference of 2 mass units with the [M + H]+ peak of the Δ6-[4-14C]T-pentapeptide conjugate.

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ACCESSION NUMBER: 93008621 EMBASE
DOCUMENT NUMBER: 1993008621
TITLE: **Liquid chromatographic purification** and **detection** of anabolic compounds.
AUTHOR: Van Ginkel L.A.; Jansen E.H.J.M.; Stephany R.W.; Zoontjes P.W.; Schwillens P.L.W.J.; Van Rossum H.J.; Visser T.
CORPORATE SOURCE: Laboratory for Residue Analysis, Nat Inst of Publ. Hlth/Env. Protect., P.O. Box 1,3720 BA Bilthoven, Netherlands

SOURCE: Journal of Chromatography, (**1992**) 624/1-2 (389-401).
ISSN: 0021-9673 CODEN: JOCRAM
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 029 Clinical Biochemistry
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The role of **liquid chromatography** within methods of **analysis** for steroids, related compounds and β-agonists in biological samples is discussed. Special attention is given to the application of **liquid chromatography** in sample preparation and extract clean-up. Different forms of **liquid chromatography**, including immunoaffinity chromatography, are compared and evaluated. Methods for confirmation based on gas chromatography-**mass spectrometry** and cryotrapping Fourier transform infrared spectrometry are discussed.

L31 ANSWER 39 OF 49 EMBASE
on STN
ACCESSION NUMBER: 91048195 EMBASE
DOCUMENT NUMBER: 1991048195
TITLE: Derivatization and gas chromatographic-**mass spectrometric detection** of anabolic steroid residues isolated from edible muscle tissues.
AUTHOR: Daeseleire E.; De Guesquiere A.; Van Peteghem C.
CORPORATE SOURCE: Laboratory of Food Analysis, State University, Harelbekestraat 72,B-9000 Ghent, Belgium
SOURCE: Journal of Chromatography - Biomedical Applications, (**1991**) 562/1-2 (673-679).
ISSN: 0378-4347 CODEN: JCBADL
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
052 Toxicology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A method was developed for the **detection** of anabolic steroid residues in edible muscle tissues. After enzymic digestion of the tissue and **purification** on disposable C18 solid-phase extraction columns, the extract was injected onto a C18 reversed-phase high-performance **liquid chromatographic** column. Three fractions or windows were collected, each containing specific **analytes.** After evaporation to dryness, the residues were subjected to a derivatization procedure which yielded suitable derivatives. After gas chromatographic-**mass spectrometric analysis**, both gas chromatographic retention data and **mass spectral** data were used for the **detection** and identification of **nortestosterone**, **testosterone**, estradiol, ethinylestradiol, trenbolone, **methyltestosterone**, chlormadinone acetate, medroxyprogesterone acetate and megestrol acetate.

L31 ANSWER 40 OF 49 EMBASE
on STN
ACCESSION NUMBER: 91131892 EMBASE
DOCUMENT NUMBER: 1991131892
TITLE: Combined high-performance **liquid**

chromatography and radioimmunoassay for the screening of 19-**nortestosterone** and **methyltestosterone** residues in meat samples.
AUTHOR: Daeseleire E.; De Guesquiere A.; Van Peteghem C.
CORPORATE SOURCE: Laboratory of Food Analysis, Fac. of Pharmaceutical Sci., State University of Ghent, Harelbekestraat 72,B-9000 Ghent, Belgium
SOURCE: Journal of Chromatography - Biomedical Applications, (**1991**) 564/2 (445-449).
ISSN: 0378-4347 CODEN: JCBADL
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A procedure is described for the **detection** of 17β-19-**nortestosterone** (17β-19-NT) and 17α-**methyltestosterone** (17α-MT) in muscle tissue by a combination of high-performance **liquid chromatography** and radioimmunoassay. The steroids were released from the muscle tissue by enzymic digestion, and the extracts were **purified** by solid-phase extraction. A meat sample from the retail trade, which was proved to contain 17β-19-NT and 17α-MT residues by gas chromatography-**mass spectrometry**, and blank meat samples obtained from non-treated experimental animals were **purified** and the extracts injected into the **liquid chromatograph.** With an automatic fraction collector, fifteen fractions of 1 ml each were obtained, which after evaporation were subjected to a radioimmunoassay for the steroid concerned. It was demonstrated that none of the fractions from the blank meat samples contains any substance that interfered with the immunochemical **detection** system. In addition, a good qualitative agreement between the two sets of results was obtained. Although the sample preparation step is rather labour-intensive, the method can be successfully applied as a reliable confirmation method for positive radioimmunoassay screening results in circumstances where no gas chromatography-**mass spectrometry** facilities are available.

L31 ANSWER 41 OF 49 EMBASE
on STN
ACCESSION NUMBER: 91131888 EMBASE
DOCUMENT NUMBER: 1991131888
TITLE: **Detection** of 19-**nortestosterone** and its urinary metabolites in miniature pigs by gas chromatography - **Mass spectrometry.**
AUTHOR: Debruyckere G.; Peteghem C.
CORPORATE SOURCE: Laboratory of Food Analysis, Fac. of Pharmaceut. Sciences, State University of Ghent, Harelbekestraat 72,9000 Ghent, Belgium
SOURCE: Journal of Chromatography - Biomedical Applications, (**1991**) 564/2 (393-403).
ISSN: 0378-4347 CODEN: JCBADL
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The metabolism of 19-**nortestosterone** was investigated in a

miniature non-castrated male pig (boar), in a castrated pig (barrow) and in a female pig (sow). Urine samples were taken before and at regular intervals after the injection of 100 mg of Laurabolin (**nortestosterone** laurate). The sample clean-up consists of preliminary solid-phase extraction, followed by high-performance **liquid chromatographic purification** and fractionation. Finally, gas chromatography-**mass spectrometry** is used to identify the 19-**nortestosterone** metabolites.

L31 ANSWER 42 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 10

ACCESSION NUMBER: 1991:90218 BIOSIS
DOCUMENT NUMBER: PREV199191049108; BA91:49108
TITLE: IDENTIFICATION OF AROMATIZABLE ANDROGENS SECRETED BY ISOLATED THECAL CELL LAYERS FROM VITELLOGENIC OVARIAN FOLLICLES OF A TELEOST AMAGO SALMON ONCORHYNCHUS-RHODURUS.
AUTHOR(S): ADACHI S [Reprint author]; KAJIURA H; KAGAWA H; NAGAHAMA Y
CORPORATE SOURCE: LAB REPRODUCTIVE BIOL, NATL INST BASIC BIOL, OKAZAKI 444, JPN
SOURCE: Biomedical Research (Tokyo), (**1990**) Vol. 11, No. 5, pp. 359-363.
CODEN: BRESD5. ISSN: 0388-6107.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 11 Feb 1991
Last Updated on STN: 12 Feb 1991

AB Aromatizable androgens, which are converted to estradiol-17β in granulosa cell layers, secreted during vitellogenesis by thecal cell layers of the teleost, amago salmon (Oncorhynchus rhodurus), were **purified** and chemically identified. Isolated thecal cell layers from vitellogenic ovarian follicles were incubated for 18 h in Ringer medium with chum salmon gonadotropin. Aromatizable androgens in samples (residues) at various steps of **purification** were **detected** by an in vitro estradiol-17β production assay using isolated granulosa cell layers from vitellogenic ovarian follicles of amago salmon. After ether extraction from the incubation medium and partition of the extract with equal volumes of 50% methanol and n-hexane, the 50% methanol phase was fractionated (27 separate fractions) by reversed-phase high-performance **liquid chromatography** and further **analyzed** by thin-layer chromatography and **mass spectrometry.** The aromatizable androgen in fractions 12 and 13 was identified as androstenedione, and that in fractions 18 and 19 as **testosterone.** The amount of **testosterone** secreted by thecal layers was about three times that of androstenedione. The present study, together with previous reports, indicates that thecal cell layers of vitellogenic ovarian follicles of amago salmon, under the influence of gonadotropin, secrete aromatizable androgens, **testosterone** as the major precursor steroid and androstenedione as the other effective one, that are converted to estradiol-17β by granulosa cell layers where the aromatase enzyme is localized.

L31 ANSWER 43 OF 49 JICST-EPlus COPYRIGHT 2005 JST on STN

ACCESSION NUMBER: 880461057 JICST-EPlus
TITLE: **Determination** of 5A-androstane-3A, 17B-diol in human serum by GC-SIM and influence of age-associated change.
AUTHOR: FUKABORI YOSHITATSU

CORPORATE SOURCE: Gunma Univ., School of Medicine
SOURCE: Nippon Naibunpi Gakkai Zasshi (Folia Endocrinologica Japonica), (1988) vol. 64, no. 5, pp. 340-353. Journal Code: F0915A (Fig. 12, Tbl. 2, Ref. 33)
CODEN: NNGZAZ; ISSN: 0029-0661
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
LANGUAGE: Japanese
STATUS: New

AB 5A-Androstane-3A, 17B-diol(A3Adiol) is a potent androgen, and is an end product of **testosterone.** Many authors measured A3Adiol levels in human plasma by various methods, but the levels of this steroid were very dissimilar. In order to validate such values, it was measured by gad chromatography - selected ion monitoring(GC-SIM) in this study. A3Adiol, 5A-Androstane-3B, 17B-diol(A3Bdiol) and **Testosterone**(T) in human peripheral serum were measured by GC-SIM at the same time. The TFA-derivatives of these compounds were **analyzed** after **purification** of the serum extract by Sephadex LH-20 microcolumn chromatography. The sensitivity was good: (16.7pg/ml: A3Adiol, 26.7pg/ml: A3Bdiol). The precision(CV=2.75%: A3Adiol, 3.11%: A3Bdiol) and the accuracy were better than ever reported. Serum A3Adiol was measured in 131 healthy men aged 15-81 years and 5 healthy women aged 25-60 years. There were remarkable differences between individuals in the serum levels of A3Adiol, but the levels in male serum(>20y) showed a significant negative correlation with age($r=-0.560$, $p<0.01$). When these healthy men were classified into three age groups of 20-39, 40-59 and 60-79 years, the values(mean±SD) for serum A3Adiol were 189.3±77.7(n=20), 127.9±59.5(n=28), and 94.9±52.9(n=73)pg/ml, respectively. There were significant differences between the levels of this steroid in all age groups($p<0.01$). There was a weak but significant correlation between serum A3Adiol and T levels($r=0.3235$, $p<0.01$) in healthy men(25-77 years, n=77). **Determination** of serum A3Adiol was influenced by age. The number of samples strongly influenced the decision of mean value of A3Adiol levels. These results suggested that these factors had to be made obvious when this steroid was studied.(author abst.)

L31 ANSWER 44 OF 49 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 86251277 EMBASE
DOCUMENT NUMBER: 1986251277
TITLE: Unsaturated fatty acids as endogenous inhibitors of tamoxifen binding to anti-oestrogen-binding sites.
AUTHOR: Hwang P.L.H.
CORPORATE SOURCE: Department of Physiology, National University of Singapore, Singapore 0511, Singapore
SOURCE: Biochemical Journal, (**1986**) 237/3 (749-755).
CODEN: BIJOAK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal
FILE SEGMENT: 037 Drug Literature Index
029 Clinical Biochemistry
010 Obstetrics and Gynecology
LANGUAGE: English

AB It is known that triphenylethylene anti-oestrogens such as tamoxifen bind to specific high-affinity anti-oestrogen-binding sites, which are distinct from oestrogen receptors. These binding sites are widely distributed in human and animal tissues, but their function and endogenous ligands are unknown. By using [3H]tamoxifen and a rat liver microsomal fraction, a

radio-ligand-binding assay was developed in an attempt to identify endogenous ligands for the anti-oestrogen-binding sites in the rat. An ether extract of rat serum inhibited [3H]tamoxifen binding to rat liver binding sites in a dose-dependent manner. Identification of the active serum constituents that inhibited [3H]tamoxifen binding was achieved by g.l.c.-**mass spectrometry** after preliminary **purification** of a rat serum extract by silica-gel t.l.c. Three unsaturated fatty acids (oleic, linoleic and arachidonic) accounted for about 50% of the total inhibiting activity of the serum extract. The concentrations of these fatty acids required to inhibit [3H]tamoxifen binding were in the range of 10-100 μM, comparable with those found in the rat circulation under physiological conditions. Saturated fatty acids present in rat serum (palmitic and stearic) did not inhibit [3H]tamoxifen binding. A survey of other fatty acids revealed that, in general, unsaturated fatty acids were far more potent than saturated fatty acids in inhibiting [3H]tamoxifen binding. These studies demonstrate that unsaturated fatty acids are quantitatively the most important circulating inhibitors of [3H]tamoxifen binding to the anti-oestrogen-binding sites. The biological significance of their interaction with these sites, however, remains to be clarified.

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ACCESSION NUMBER: 86196464 EMBASE
DOCUMENT NUMBER: 1986196464
TITLE: Resolution of mouse hepatic cytochrome P-450 isozymes by chromatofocusing.
AUTHOR: Marriage H.J.; Harvey D.J.
CORPORATE SOURCE: University Department of Pharmacology, Oxford OX1 3QT, United Kingdom
SOURCE: Journal of Chromatography, (**1986**) VOL. 354/- (383-392).
CODEN: JOCRAM
COUNTRY: Netherlands
DOCUMENT TYPE: Journal
FILE SEGMENT: 037 Drug Literature Index
029 Clinical Biochemistry
030 Pharmacology
LANGUAGE: English

AB Constituent cytochrome P-450 isozymes from mouse hepatic microsomes were fractionated by chromatofocusing on Polybuffer Exchanger 94 over the pH range 5.3-8.3 and characterized by polyacrylamide gel electrophoresis. Eight isozymes were **detected** in fractions from unpretreated mice and seven from phenobarbitone-pretreated animals. Isozyme-containing fractions were reconstituted and shown to produce specific monohydroxy metabolites from both **testosterone** and Δ9-tetrahydrocannabinol.

L31 ANSWER 46 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 11

ACCESSION NUMBER: 1986:151191 BIOSIS
DOCUMENT NUMBER: PREV198681061607; BA81:61607
TITLE: **ANALYSIS** OF PROFILES OF UNCONJUGATED STEROIDS IN RAT TESTICULAR TISSUE BY GAS CHROMATOGRAPHY-**MASS SPECTROMETRY.**
AUTHOR(S): ANDERSSON S H G [Reprint author]; SJOVALL J
CORPORATE SOURCE: DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, KAROLINSKA INSTITUTET, BOX 60 400, S-104 01 STOCKHOLM, SWEDEN
SOURCE: Journal of Steroid Biochemistry, (**1985**) Vol. 23, No. 4, pp. 469-476.

CODEN: JSTBBK. ISSN: 0022-4731.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 25 Apr 1986
Last Updated on STN: 25 Apr 1986

AB A gas chromatographic-**mass spectrometric** (GC-MS) method for **analysis** of unconjugated steroids in a rat testis is described. A combined solvent-solid extraction procedure, utilizing Lipidex 1000 and Sep-Pak C18, gives a 25-fold **purified** extract. Steroids in this extract are fractionated by straight phase high-performance **liquid chromatography** (HPLC) on a LiChrosorb DIOL column in n-hexane-2-propanol, 92:8 (v/v). Four fractions are collected and the steroids are converted to tert-butyldimethylsilyl (TBDMS), 3-enol-TBDMS, and mixed TBDMS-trimethylsilyl (TMS) derivatives using TBDMS- and TMS-imidazole with sodium formate as catalyst under conditions suitable for the steroids present in the respective fractions. The derivatives are **purified** by reversed phase HPLC in 100% methanol and are **analyzed** by GC-MS, using selected ion monitoring of the major ions of high mass. For quantification, a mixture of known amounts of ten 14C-labeled steroids, [3H]estradiol and [2H3]estradiol are added to the testis homogenate. The mean concentrations (ng g wet wt) of the twelve steroids **determined** were: 4-androstene-3,17-dione, 4.0; **testosterone**, 127; 17β-hydroxy-5α-androstan-3-one, 4.5; 5α-androstane-3α,17β-diol, 5.7; 5α-androstane-3β,17β-diol, 1.5; progesterone, 5.5; 17α-hydroxyprogesterone, 14.4; 3β-hydroxy-5-androsten-17-one, 0.07; 5-androstene-3β,17β-diol, 0.25; 3β-hydroxy-5-pregnen-20-one, 10.3; 3β,17β-dihydroxy-5-pregnen-20-one, 0.95; and estradiol, 0.025. Variations between animals were large whereas testes from the same animal in most cases had similar steroid concentrations.

L31 ANSWER 47 OF 49 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 1985:309014 BIOSIS
DOCUMENT NUMBER: PREV198579089010; BA79:89010
TITLE: TRIAMCINOLONE ACETONIDE-21-OIC-ACID METHYL ESTER A POTENT LOCAL ANTIINFLAMMATORY STEROID WITHOUT **DETECTABLE** SYSTEMIC EFFECTS.
AUTHOR(S): GORSLINE J [Reprint author]; BRADLOW H L; SHERMAN M R
CORPORATE SOURCE: DEP PATHOL, MIRIAM HOSP, 164 SUMMIT AVE, PROVIDENCE, RI 02906, USA
SOURCE: Endocrinology, (**1985**) Vol. 116, No. 1, pp. 263-273.
CODEN: ENDOAO. ISSN: 0013-7227.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB Esters of the 21-oic acid of triamcinolone acetonide (TA, 9α-fluoro-11β,16α,17α,21-tetrahydroxy-pregna-1,4-diene-3,20-dione 16,17-acetonide), a potent synthetic glucocorticoid, were synthesized in both tritiated and unlabeled forms. The synthesis involves oxidation to the 21-dehydro compound with methanolic cupric acetate, further oxidation to the acid with methylene blue in the presence of KCN at pH 6.5, esterification with diazomethane in the presence of methanol or ethanol, to produce the methyl ester of TA (TAme) or ethyl ester, respectively, and **purification** of the products by TLC and HPLC [high performance **liquid chromatography**]. The MW and structures of the esters were established by **mass**

spectrometry and nuclear magnetic resonance. The binding of [3H]TAme to steroid receptors or serum steroid-binding proteins and the in vitro hydrolysis of the ester were evaluated simultaneously, by chromatography on Sephadex LH-20 columns in aqueous buffer. [3H]TAme is bound with high affinity by receptors from human leukemic cells and rat liver. The pattern of competition for this binding is characteristic of glucocorticoid receptors: TA ≈ TAme > R5020 (a synthetic progestin) ≈ aldosterone > 5α- **dihydrotestosterone.** [3H]TAme is not bound **detectably** by serum steroid-binding proteins and is rapidly hydrolyzed during incubation with serum at 37° C. The acidic product has a very low affinity for the glucocorticoid receptor. Complexes of [3H]TAme with human and rat receptors have sedimentation coefficients of 9-10 S in hypotonic buffer containing 20 mM Na2MoO4 and .apprx. 4S in hypertonic, molybdate-free buffer. These values of sedimentation coefficient are similar to those of the oligomeric and monomeric forms, respectively, of the same receptors labeled with [3H]TA, and of mammalian steroid receptors, in general. The antiinflammatory activity of TAme in rats is comparable to that of prednisolone, but the ester is devoid of the side effects associated with prednisolone treatment (suppression of thymic weight and of serum corticosterone concentration). Thus, TAme may eventually be useful clinically, as a local antiinflammatory drug.

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ACCESSION NUMBER: 1984:257846 BIOSIS
DOCUMENT NUMBER: PREV198477090830; BA77:90830
TITLE: STEROIDS IN PORCINE FOLLICULAR FLUID **ANALYSIS** BY **LIQUID CHROMATOGRAPHY** CAPILLARY GAS CHROMATOGRAPHY AND CAPILLARY GAS CHROMATOGRAPHY **MASS SPECTROSCOPY** AFTER **PURIFICATION** ON SEP-PAK C-18 AND ION EXCHANGE CHROMATOGRAPHY.
AUTHOR(S): KHALIL M W [Reprint author]; LAWSON V
CORPORATE SOURCE: MRC GROUP REPRODUCTIVE BIOLOGY, UNIV WESTERN ONTARIO, 339 WINDERMERE RD, LONDON, ONT, CAN N6A 5A5
SOURCE: Steroids, (**1983**) Vol. 41, No. 4, pp. 549-567. CODEN: STEDAM. ISSN: 0039-128X.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB Steroids in porcine follicular fluid have been concentrated by reverse phase chromatography on SEP-PAK C18 and **purified** further on the cation exchanger SP- Sephadex C-25. Fractionation into unconjugated neutral and phenolic steroids, glucuronides and sulfates was carried out on triethylaminohydroxypropyl Sephadex LH-20 (TEAP-LH-20). The unconjugated neutral fraction was **analyzed** by high pressure **liquid chromatography** (HPLC) on a C18 radial cartridge 5 mm I.D.; 10 μ, or on a C18 5 μ RESOLVE column, and by capillary gas chromatography (GC) on a 12 M OV-1 cross linked fused silica column. **Testosterone**, progesterone and androstenedione were the major steroids **detected** by HPLC monitored at 254 nm, although 17-hydroxy-, 20α-dihydro- and 20β-dihydroprogesterone were also present. Pregnenolone, pregnanediol, dehydroepiandrosterone, 17-hydroxypregnenolone and androsterone were **detected** by capillary GC as their O-methyloxime trimethylsilylether derivatives. Further confirmation of structure was provided by complete **mass spectral** data or by selective ion monitoring (SIM).

L31 ANSWER 49 OF 49 EMBASE

on STN

ACCESSION NUMBER: 82001706 EMBASE
DOCUMENT NUMBER: 1982001706
TITLE: **Analysis** of profiles of conjugated steroids in urine by ion-exchange separation and gas chromatography-**mass spectrometry.**
AUTHOR: Axelson M.; Sahlberg B.-L.; Sjovall J.
CORPORATE SOURCE: Dept. Physiol. Chem., Karolinska Inst., S-104 01 Stockholm, Sweden
SOURCE: Journal of Chromatography, (**1981**) 224/3 (355-370).
CODEN: JOCRAM
COUNTRY: Netherlands
DOCUMENT TYPE: Journal
FILE SEGMENT: 037 Drug Literature Index
029 Clinical Biochemistry
003 Endocrinology
030 Pharmacology
LANGUAGE: English

AB A simplified, flexible method for the **analysis** of metabolic profiles of steroids in urine is described. Solid extraction with Amberlite XAD-2 or Sep-Pak C18 cartridges is followed by group fractionation of unconjugated neutral and phenolic steroids, monoglucoronides, monosulphates and disulphates on the lipophilic strong anion exchanger triethylaminohydroxypropyl Sephadex LH-20 (TEAP-LH-20). Following brief enzymatic hydrolysis or solvolysis the steroids are **purified** on TEAP-LH-20. O-Methyloxime and trimethylsilyl ether derivatives are prepared and **purified** by filtration through Lipidex 5000, and are then **analyzed** by glass capillary column gas-**liquid chromatography** and gas chromatography-**mass spectrometry.** Between 2 and 5 ml of urine are used for a comprehensive **analysis.** Unconjugated neutral and phenolic steroids are isolated in half a day, corresponding steroids in the conjugate fractions in two days. No fraction containing steroids is discarded, but the **analysis** can be limited to a selected fraction.

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L19 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:619338 HCAPLUS
DOCUMENT NUMBER: 137:348560
TITLE: The diagnosis of congenital adrenal hyperplasia in the newborn by gas chromatography/mass spectrometry analysis of random urine specimens
AUTHOR(S): **Caulfield, Michael P.**; Lynn, Thomas; Gottschalk, Michael E.; Jones, Kenneth L.; Taylor, Norman F.; Malunowicz, Ewa M.; Shackleton, Cedric H. L.; **Reitz, Richard E.**; Fisher, Delbert A.
CORPORATE SOURCE: Quest Diagnostics' Nichols Institute, San Juan Capistrano, CA, 92690, USA
SOURCE: Journal of Clinical Endocrinology and Metabolism (2002), 87(8), 3682-3690
CODEN: JCEMAZ; ISSN: 0021-972X
PUBLISHER: Endocrine Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Definitive neonatal diagnosis of congenital adrenal hyperplasia (CAH) is frequently complicated by normal 17-hydroxyprogesterone levels in 21-hydroxylase-deficient patients, residual maternal steroids, and other interfering substances in neonatal blood. In an effort to improve the diagnosis, the authors developed a gas chromatog./mass spectrometry method for simultaneous measurement of 15 urinary steroid metabolites as early as the first day of life. Furthermore, the authors developed 11 precursor/product ratios that diagnose and clearly differentiate the four enzymic deficiencies that cause CAH. Random urine samples from 31 neonatal 21-hydroxylase-deficient patients and 59 age-matched normal newborns were used in the development. Addnl., samples from two 11β-hydroxylase-deficient patients and one patient each for 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies were used. The throughput for one bench-top gas chromatog./mass spectrometry instrument is 20 samples per day. Thus, this method affords an accurate, rapid, noninvasive means for the differential diagnosis of CAH in the newborn period without the need for invasive testing and ACTH stimulation.

CC 9-1 (Biochemical Methods)
ST congenital adrenal hyperplasia newborn urine steroid GS MS; gas chromatog mass spectrometry steroid urine newborn CAH diagnosis; steroid hydroxylase insufficiency newborn steroid urine; hydroxysteroid dehydrogenase insufficiency newborn steroid urine
IT Human
Newborn
Urine analysis
(congenital adrenal hyperplasia diagnosis in newborn by gas chromatog.-mass spectrometry anal. of random urine specimens)
IT Adrenal cortex, disease
(congenital adrenal hyperplasia; congenital adrenal hyperplasia diagnosis in newborn by gas chromatog.-mass spectrometry anal. of random urine specimens)
IT Hyperplasia
(congenital adrenal; congenital adrenal hyperplasia diagnosis in newborn by gas chromatog.-mass spectrometry anal. of random urine specimens)
IT 53-05-4, Tetrahydrocortisone 68-60-0, Tetrahydro-11-deoxycortisol 516-42-7, α-Cortolone 520-88-7, 16α-Hydroxypregnenolone 570-39-8 570-52-5, 17α-Hydroxypregnanolone 603-99-6, Pregnanetriolone 667-66-3, β-Cortolone 1098-45-9, Pregnanetriol

1232-73-1, 16α-Hydroxydehydroepiandrosterone 80380-40-1
80380-41-2 82056-07-3, 5-Pregnenetriol 158722-91-9 474801-54-2
RL: ANT (Analyte); BSU (Biological study, unclassified); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(congenital adrenal hyperplasia diagnosis in newborn by gas chromatog.-mass spectrometry anal. of random urine specimens)

IT 9029-66-7 9029-67-8, Steroid 17α-hydroxylase 9029-68-9, 21-Hydroxylase 9044-85-3, 3β-Hydroxysteroid dehydrogenase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(deficiency; congenital adrenal hyperplasia diagnosis in newborn by gas chromatog.-mass spectrometry anal. of random urine specimens)

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